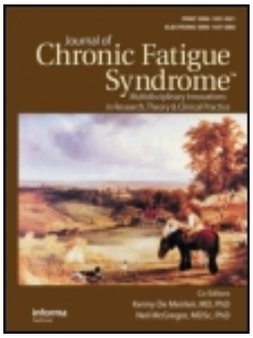

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Prevalence in the Cerebrospinal Fluid of the Following Infectious Agents in a Cohort of 12 CFS Subjects: Human Herpes Virus-6 and 8; *Chlamydia* Species; *Mycoplasma* Species; EBV; CMV; and Coxsackievirus

Susan Levine, MD

ABSTRACT. Over the last decade a wide variety of infectious agents has been associated with the chronic fatigue syndrome (CFS) as potential etiologies for this disorder by researchers from all over the world. Many of these agents are neurotrophic and have been linked previously to other diseases involving the central nervous system (CNS). Human herpes virus-6 (HHV-6), especially the B variant, has been found in autopsy specimens of patients who suffered from multiple sclerosis. Because patients with CFS manifest a wide range of symptoms involving the CNS as shown by abnormalities on brain MRIs, SPECT scans of the brain and results of tilt table testing we sought to determine the prevalence of HHV-6, HHV-8, Epstein-Barr virus (EBV), cytomegalovirus (CMV), *Mycoplasma* species, *Chlamydia* species, and Coxsackie virus in the spinal fluid of a group of 12 patients with CFS. Although we intended to search mainly for evidence of actively replicating HHV-6, a virus that has been associated by several researchers with this disorder, we found evidence of HHV-8, *Chlamydia* species, CMV and Coxsackie virus in

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6/12 samples. Attempts were made to correlate the clinical presentations of each of these patients, especially the neurological exams and results of objective testing of the CNS, with the particular infectious agent isolated. It was also surprising to obtain such a relatively high yield of infectious agents on cell free specimens of spinal fluid that had not been centrifuged. Future research in spinal fluid analysis, in addition to testing tissue samples by polymerase chain reaction (PCR) and other direct viral isolation techniques will be important in characterizing subpopulations of CFS patients, especially those with involvement of the CNS.

KEYWORDS. HHV-6, HHV-8, cerebrospinal fluid, PCR, chronic fatigue syndrome

INTRODUCTION

The chronic fatigue syndrome (CFS) is characterized by the following symptom complex: debilitating fatigue, low grade fevers, myalgias, neurocognitive difficulties, and sleep disorders (1,2). Over the last several decades researchers have implicated a number of infectious agents as potential etiologic causes (3-6) of this rather perplexing disorder because over 60% of patients report the onset of their fatigue following an acute viral infection (7). Herpesviruses (8,9), enteroviruses (5), and *Chlamydia* species (10) have also been shown to invade the central nervous system, which is probably affected in at least a subpopulation of CFS patients as shown by studies employing brain magnetic resonance imaging (MRI) (11); single photon emission computed tomographic (SPECT) scans (12,13); and results of testing involving the hypothalamic pituitary axis (14).

We sought, based on results of other researchers in this area (15,16), using PCR testing methodology performed in 2 separate laboratories, to determine the prevalence of the following infectious agents in the cerebrospinal fluid of 12 CFS subjects: human herpes virus-6; human herpes virus-8; CMV; EBV; *Mycoplasma* species; Coxsackie species; and *Chlamydia* species.

MATERIALS AND METHODS

Methods

A modified nested PCR protocol was used to detect HHV-6 DNA in 6 of the 12 plasma and cerebrospinal fluid samples from CFS patients according to the methods of Seccherio et al. (17). HHV-6 variants A and B; *Chlamydia* species; *Mycoplasma* species, EBV, CMV, HHV-8, and Coxsackie species were tested at Medical Diagnostic Laboratories (MDL) using primers for each of these infectious agents described by Relman et al. (36) in both the cerebrospinal fluid and plasma of the CFS patients. This latter method involved extraction of genetic material from lymphocytes or cerebral spinal fluid of CFS subjects; construction of the appropriate 'primers'; performance of the PCR procedure; and completion of the process by performing gel electrophoresis of the PCR product. The usual precautions against contamination of product were taken.

After obtaining informed consent, lumbar punctures were conducted under fluoroscopic guidance at East River Medical Imaging and at least 20 mL of cerebrospinal fluid was obtained. A portion of each sample was immediately shipped at room temperature (RT) to MDL laboratories or to Advanced Biotechnologies for immediate analysis. Two mililiter of sample from each patient was sent for protein, glucose, RBC and WBC determination to a local laboratory. In addition, 5 of the 12 samples were sent for Lyme Western Blot IgG and IgM determination to Stonybrook laboratories.

A Karnofsky score (Figure 1) was determined on each of the patients.

Subjects

Twelve subjects, 10 females and 2 males, from the NY, NJ and CT area who ranged in age from 25 to 57 and who fulfilled the 1994 case definition for CFS (Table 1) signed informed consent for a spinal tap.

RESULTS

Of the twelve CFS subjects tested six demonstrated a positive PCR in their spinal fluid for one of the following infectious agents: HHV-6 (patient #1); *Chlamydia* species (patients #5, 6); CMV (patient #8); HHV-8 (patient #9); and Coxsackievirus (patient #7). None of the latter six subjects who also had their plasma tested for these infectious agents had a positive result in plasma (patients #1, 7, 8, 9) (Tables 2 and 3).

FIGURE 1. Karnofsky Scale

GRADE	SCALE
100	Normal, no complaints, no evidence of disease.
90	Able to carry on normal activity, minor signs or symptoms of disease.
80	Normal activity with effort, some signs or symptoms of disease.
70	Care for self. Unable to carry on normal activities or to do active work.
60	Requires occasional assistance, but is able to care for most of his/her needs.
50	Requires considerable assistance and frequent medical care.
40	Disabled, requires special care and assistance.
30	Severely disabled, hospitalization is indicated although death not imminent.
20	Hospitalization necessary, very sick, active supportive treatment necessary.
10	Moribund, fatal processes progressing rapidly.
0	Dead

TABLE 1. Characteristics of CFS Subjects Who Underwent Plasma and Cerebrospinal Fluid Testing

Patient	Age	Sex	Date of Onset	Term of Illness	Abrupt versus Gradual Onset	Disability Status	Obtained Signed Consent Forms
1	40	F	1994	6 years	Abrupt	as of 7/00	Yes
2	39	M	1988	2 years	Gradual	as of 7/00	Yes
3	30	F	1995	5 years	Gradual	as of 10/95	Yes
4	42	M	1997	3 years	Abrupt	as of 1998	Yes
5	57	F	1994	6 years	Abrupt	as of 1994	Yes
6	25	F	1996	4 years	Abrupt	as of 07/00	Yes
7	42	F	1980	20 years	Abrupt	as of 1992	Yes
8	47	F	1994	6 years	Gradual	Not	Yes
9	52	F	1998	2 years	Gradual	as of 05/00	Yes
10	47	F	1995	5 years	Abrupt	as of 1995	Yes
11	50	F	1999	1 year	Gradual	as of 05/00	Yes
12	46	F	1993	7 years	Abrupt	as of 2000	N/A

TABLE 2. Additional Clinical Findings on CFS Subjects with Positive Cerebrospinal Fluid Results by PCR

Patient	Age	Sex			Clinical History	
1	40	F	–	8/94	Hypesthesia to pin over right V1 and V2 dermatomes of 5th cranial nerve; right facial paresthesia and hypesthesia	+HHV-6
			–	1995	Severe symptoms recede	
			–	5/00	Elevated cholesterol level of 300	
			–	5/00	Sleep Study with normal outcome	
			–	5/00	EKG with incomplete right bundle branch blockage	
			–	5/00	EBCT showing mildly dense calcification	
5	57	F	–	1/97	MRI of Brain with abnormal findings (question of MS)	+ Chlamydia
			–	12/97	MRI of Brain with no significant change since 1/97	
			–	12/97	Visual Evoked Potentials–disturbance of the prechiasmatic visual pathways bilaterally	
			–	12/97	Somatosensory Evoked Potentials–normal results	
			–	12/97	Brainsem Auditory Evoked Potentials–normal results	
6	25	F	–	1996	Diagnosed with fibromyalgia following 2 ER visits for pain	+ Chlamydia
			–	12/00	MRI of Brain with negative findings	
7	42	F	–	12/80	Toxic exposure (Ammonia Gas Inhalation) followed by pneumonitis and mono-like illness	+ Coxsackie Virus
			–	1991	Adult onset diabetes mellitus –C-peptide level of 1.38ng/ml –Free thyroxine index of 3.1 –TSH level of 7.4 microunits/ml	
			–	11/91	Hypothyroidism	
			–	3/92	HCM necrotic mass removed from lower abdomen	
			–	1993	Necrotic tissue removed from right buttocks. Low dehydroepiandrosterone level	
			–	1994	2 hospitalizations for coma-related diabetic ketoacidosis	
			–	1996	MRI of face–Masses in both parotid glands (f/u in 1998) Enlarged lymph nodes, TMJ, frozen left shoulder, and Severe cognitive disfunction	
			–	2/98	Spect Scan with minimally decreased perfusion to the frontal lobes bilatterally improvement from '97 study	
			–	3/99	–Hospitalized with diabetic ketoacidosis. –Mild mitral regurgitation. –High coxsackie B IgG, Serum type 1, 3, 5 & 6 –High coxsackie A IgG, Serum type 4, 9, & 16	
			–	12/00	High glucose (104) in CSF	
8	47	F	–	1980	Osteoarthritis	+CMV
			–	1989	Hysterectomy for bleeding fibroids	
			–	08/94	MRI cervical spine showing mild diffuse bulging C4-C5	
			–	09/94	"Transient" thrombocytopenia	
			–	09/94	EMG left upper extremity, shoulder impingement	
			–	12/94	Negative Lupus Anticoagulant	
			–	12/94	Anti-cardiolipin IgM of 13	
			–	12/94	Anti-platelet Ab-IgG of 32	
			–	12/99	Coronary catheterization–'spasm' of vessels	
			–	12/99	MRI of brain showing 'mini-strokes'	
9	52	F	–	'97&'98	2 heart stents due to coronary artery obstruction	+HHV-8
			–	1998	Diagnosed with fibromyalgia (diffuse muscle pain)	
			–	9/99	C-Reactive protein level of 6.3 (0-5) MG/L	
			–	3/00	Nuclear stress test–normal findings	
			–	4/00	MRI of cervical spine–large disc/osteophyte 'occluding subarachnoid space' at C4/C5	
			–	6/00	EMG–negative	
			–	6/00	Dysphagia–smoker, having difficulty swallowing	
			–	12/00	MRI of brain and EEG–negative; experiencing cognitive decline and loss of balance	
			–	12/00	Abdominal/pelvic sonagram–finding of complex left ovarian cyst (11mm)	
			–	12/00	Osteopenic in lumbar spine, bone density testing	
			–	1/01	HIV negative by ELISA	

TABLE 3. Results of Plasma and Cerebrospinal Fluid Testing of Twelve CFS Subjects by PCR for: HHV-6, HHV-8, EBV, *Chlamydia* Species, *Mycoplasma* Species, CMV, and Coxsackie Virus Species

Patient (No., Age, Sex)	HHV-6 (+/−) CSF	HHV-6 (+/−) Plasma	EBV CSF	EBV Plasma	Chlamydia CSF	Chlamydia Plasma	CMV CSF	CMV Plasma	HHV-8 CSF	HHV-8 Plasma	Coxsackie CSF	Coxsackie Plasma	Protein	Glucose	Lyme (3)	Opening Pressure (4)	Karnofsky Score (5)	MRI of the Brain	SPECT of the Brain
1,40,F (1,2)	+	−	−	−	−	−	−	−	−	−	−	−					80	−	
2,39,M (1)	−	−	−	−	−	−	−	−	−	−	−	−	27	65		150	40		
3,30,F (1,2)		−		−		+		−	−	−	−	−	17	55	−		50	−	+
4,42,M (1)		−		−			−	−	−	−	−	−	28	58			50		−
5,57,F (1)	−		−		$\boxed{+}$		−		−	−	−	−	29	59	−	60	60	+	
6,25,F (1)	−	+	−		$\boxed{+}$		−		−	−	−	−	46	58		11	40		
7,42,F (1)	−	−	−	−	−	−	−	−	−	−	$\boxed{+}$	−	30	$\boxed{104}$	−	10	45		
8,47,F (1,2)	−	−	−	−	−	−	$\boxed{+}$	−	−	−	−	−	45	52	−		70	+	
9,52,F (1,2)	−	−	−	−	−	−	−	−	$\boxed{+}$	−	−	−	44	59			50	−	
10,47,F (1,2)	−	−	−	−	−	−	−	−	−	−	−	−	38	63	−		40		
11,50,F (1)	−	−	−	−	−	−	−	−	−	−	−	−	21	63			40		
12,46,F (1,2)	−	+							−	−	−	−					40		−

(1 and 2) See Methodology Section
(3) ELISA LYME TEST
(4) cm's of water
(5) See Karnofsky Scale

Patient #1 who reported an abrupt onset, had abnormal neurological findings on clinical exam 5 years ago when she first became ill, suggestive of trigeminal neuralgia. Although she has become disabled for the last 6 months, prior to that she was able to function almost normally and her overall KS score is '80.'

Patient #5 also had an abrupt onset and has remained disabled for the last 5 years. Her KS score is '60.' She also demonstrated an abnormal MRI of the brain with UBOs (unidentified bright objects) in the frontal cortex but has had a normal neurological exam recently. She had slightly abnormal VEPs with normal Somatosensory and Brainstem Auditory Evoked Potentials when last tested 3 years ago.

Patient #6 has had predominantly symptoms of fibromyalgia (FM) without clinically apparent neurological findings. Her KS score is '75.'

Patient #7 has been disabled for 20 years. Her illness was triggered by ammonia gas inhalation following a nuclear accident. She subsequently developed thyroiditis, insulin dependent diabetes mellitus, necrotizing granulomata of the lymph nodes, parotid glands and abdominal wall, and had an abnormal SPEC scan of the brain 2 years ago. Her KS score is '45.'

Patient #8 continues to work and has an abnormal MRI of the brain which demonstrates evidence of 'mini strokes' as well as positive anticardiolipin and antiplatelet IgG.

Finally, Patient #9 has been disabled for 8 months and has had stents placed in her coronary arteries. Her HIV test was negative as of January 2001.

DISCUSSION

We isolated several different infectious agents from our CFS patients by using nested PCR testing on samples of their cerebrospinal fluid. The two methods used differed in that the one employed by Advanced Biotechnologies involved short-term co-culture of patient's cells with human cord blood cells or fibroblasts (17). Although this technique is considered more 'sensitive,' the lack of positive results for HHV-6 by PCR testing using this method may be explained by the low amount of infectious cell-free virus being released into the cerebrospinal fluid.

Another reason for the low rate of isolation of HHV-6 from the cerebrospinal fluid in our patients may be that none exhibited 'gross' neurological findings at the time of testing as did at least one patient reported in the literature with a detectable cerebrospinal viral load (15).

Interestingly, patient #1, from whose cerebrospinal fluid HHV-6 was isolated was found to have some sensory findings at the time of onset of her illness, which was abrupt, five years ago. This presentation seemed compatible with a type of trigeminal neuralgia. She continues to experience intermittent facial tingling and numbness. Her brain MRI is negative.

Patient #5 who demonstrated *Chlamydia* species by PCR testing of her cerebrospinal fluid was at one time given the clinical diagnosis of multiple sclerosis (MS) based on an 'abnormal' MRI of the brain. However, she had a normal neurological exam and absent oligoclonal bands on a prior spinal fluid analysis.

Chlamydia species have been isolated from patients with MS but mainly those with highly progressive disease (18). One patient with MS described by these authors, who was on a spiraling downhill course despite several trials of immunosuppressive agents improved markedly after a course of antibiotics. This group was also able to isolate, however, *Chlamydia* species from patients with other inflammatory CNS disorders but not from normal controls.

Chia et al. (10) describe a cohort of 10 CFS patients who demonstrated elevated serum antibody titers to *Chlamydia* species but who did not have obvious neurological symptoms. Their fatigue also responded somewhat to a course of antibiotic therapy.

The second patient from whom we isolated *Chlamydia* in the spinal fluid, patient #6, did not have significant clinical findings on neurological exam; she had a normal MRI of the brain.

Other groups of researchers failed to isolate this organism by PCR testing of brain tissue from MS plaques (20) or from the plasma of patients with CFS (21).

Patient #7 had Coxsackievirus species DNA isolated from the cerebrospinal fluid. Enteroviral sequences have been previously reported in the stool (22), muscle biopsies (23), and serum (24) of patients with CFS. Of interest is that this patient's symptoms began soon after an ammonia gas exposure. Eleven years later she developed insulin dependent diabetes mellitus, which at least in mice has been shown to occur by local infection with Coxsackievirus (25,26). Infection by this group of organisms has been shown to lead to inflammation and local tissue damage, probably through induction of a type of 'autoimmune' state.

This patient was also found to have elevated levels of both Coxsackie A serotypes 4, 9, and 12 IgG and Coxsackie B serotypes 1, 3, 5 and 6 IgG in the serum. A similar mechanism of 'self' destruction of tissue cells has been postulated to occur in patients with autoimmune thyroiditis

and may have also contributed to the formation of granulomata in this patient's parotid gland, lymph nodes and abdominal wall. Isolation of Coxsackie virus RNA sequences directly from tissue specimens using PCR technology and serotyping employing restriction endonuclease assays, may be able to support this hypothesis.

Patient #8 demonstrated CMV in the cerebrospinal fluid. Although one group has isolated CMV in the cardiac muscle of subjects diagnosed with CFS (27), there have been no published reports of its direct isolation from cerebrospinal fluid in patients with this disorder.

CMV has also been associated with coronary heart disease in patients following cardiac transplant (28). Of interest is that this patient underwent cardiac catheterization for a complaint of chest pain with a finding of 'coronary vasospasm' and presumably the absence of atherosclerotic plaques. She did, however, demonstrate elevated cardiolipin IgM, as well as antiplatelet IgG in the serum. Her lupus anticoagulant was negative. Finally, an MRI of the brain showed some 'mild ischemic disease.'

Patient #9 had HHV-8 isolated from her cerebrospinal fluid. The latter finding is surprising in light of the fact that two prior groups failed to isolate this virus from the plasma of a large group of patients with CFS (29,30), and the rate of isolation of cell free virus from the cerebrospinal fluid is expected to be quite low.

The actual epidemiology of HHV-8 remains unchartered and the available assays may be unreliable. Mainly, this virus has been isolated from patients with Kaposi's sarcoma; those with the endemic variety of this cancer who are HIV seronegative have a ten fold higher yield of HHV-8 in their affected tissue than those who are HIV seropositive (31). Our patient was HIV seronegative.

In summary, although we failed to test the cerebrospinal fluid of normal healthy donors, other groups have been able to detect HHV-6 DNA in only a small percentage of normal controls (15). Because of the myriad of published findings involving the central nervous system in patients with CFS, including the presence of 'unidentified bright objects' on MRIs of the brain; multifocal perfusion defects on SPECT Scans of the brain; autonomic dysfunction suggested by the finding of neurally-mediated hypotension; and the likely interaction between the neuroendocrine and cytokine systems, it is likely that neurotrophic infectious agents play some role, as both triggers and perpetuators, in the complex pathophysiology of this disorder (14,32,33).

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