

Outbreak of aseptic meningitis among adults in southern Taiwan

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A total of 38 adult cases of aseptic meningitis were diagnosed based on clinical manifestations as well as examination of cerebrospinal fluid at Kaohsiung Veterans General Hospital in 2001. The majority (31 cases, 82%) of cases occurred between June and August, and most (33 cases, 87%) of them aged from 18 to 35 years (median age, 25 years). The male-to-female ratio was 2.16:1. Common clinical presentations included headache, fever, nausea or vomiting, and symptoms of common cold. On initial cerebrospinal fluid examination, white cell counts were above 500 cells/mm³ in 7 (18%) cases, and neutrophils predominated in 10 (28%) of 36 cases. The cerebrospinal fluid protein concentration was below 100 mg/dL in 29 (77%) of 38 cases, and hypoglycorrhachia (cerebrospinal fluid/serum glucose ratio <0.5) was seen in 9 (24%) cases. Enterovirus as a definite etiology was confirmed by isolation from cerebrospinal fluid in 17 (45%) of 38 cases and was the presumptive etiology by isolation from the stool or throat swab in 2 (5%) cases. Of the 19 cases with positive viral isolation, echovirus serotype 30 accounted for the majority (15 cases, 79%), followed by echovirus serotype 6 (3 cases, 16%), with one (5%) case undetermined. The viral isolation rate from cerebrospinal fluid correlated to the day of cerebrospinal fluid sampling after disease onset ($\chi^2 = 12.05$, $p=0.007$). All patients were discharged uneventfully without receiving antibiotic therapy.

Keywords: Aseptic meningitis, adult, echovirus, outbreak, Taiwan

Viral infections of the central nervous system (CNS) have 2 major clinical presentations, aseptic meningitis and meningoencephalitis. Aseptic meningitis is by far the most frequent, and enteroviruses are the major etiologies (35%-83%) [1]. Enteroviral meningitis is usually a benign clinical entity. Enteroviral epidemics are common in infants and young children, and most reports of enteroviral meningitis are associated with pediatric patients worldwide as well as in Taiwan [2-4]. In the summer of 2001, a cluster of aseptic meningitis in adults was observed at Kaohsiung Veterans General Hospital (KSVGH), where patients admitted are mostly from southern Taiwan. A total of 38 cases were diagnosed in 2001 compared with only 5 cases in 2000. Enteroviral meningitis confirmed by viral isolation from the cerebrospinal fluid (CSF) accounted for about one half of cases. The purpose of this report was to describe the clinical epidemiology and manifestations, laboratory findings, and the outcome of viral culture.

Materials and Methods

We studied the medical records of 38 patients older than 15 years admitted to KSVGH between January 1, 2001 and December 31, 2001 with suspected aseptic meningitis, and 253 adults whose specimens of CSF were submitted for viral culture were reviewed. Kaohsiung Veterans General Hospital is a 1266-bed teaching hospital that has 42 beds for treatment of patients with infectious disease, 35 beds for neurologic disease, and a reference microbiologic laboratory. Most data were obtained from the records of the infectious disease and neurology departments and the microbiology laboratory. International classification of disease (ICD) numbers were coded for viral (047.9) or unspecified meningitis (322.1).

A diagnosis of aseptic meningitis was made based on clinical findings of CSF pleocytosis and/or positive viral isolation from the CSF without evidence of bacteria, fungus, tuberculosis, parasite by smear, serology test, or culture of the CSF. Cerebrospinal fluid pleocytosis was defined as the presence of more than 5 white blood cells (WBC) per cubic millimeter (≥ 5 cells/mm³). Patients were excluded if they presented with features of encephalitis, had CSF findings interpreted

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to be a result of traumatic tapping [5], or had a diagnosis that might be associated with other etiologies [6,7] such as autoimmune disease, drug or toxin exposure, vaccination, trauma, organic lesions of CNS, and malignancy by follow-up.

The information collected for these 38 patients included age, sex, date of disease onset, clinical signs and symptoms, complete blood counts and serum glucose, CSF protein, cell counts, glucose, as well as management and outcome. All patients were discharged uneventfully without receiving empirical antibiotic chemotherapy.

Throat and rectal swabs were collected in 2 mL of viral collection media (mixture of Hanks balanced salt solution, bovine serum albumin 7.5% solution, HEPES [N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid] 1 M solution and antibiotic with penicillin [10^4 U/mL], streptomycin [10 μ g/mL], and amphotericin B [0.025 μ g/mL]). Suspensions through 0.45 mm filter were inoculated onto a tube coated with continuous cell lines.

Tube cultures of continuous monkey kidney cells (MK-2, Vero), human embryonic lung fibroblasts (HEL), rhabdomyosarcoma cells (RD), human epidermoid carcinoma cells (HEp-2), and dog kidney cells (MDCK) were used for viral isolation. All cultures were incubated at 35°C and observed daily for cytopathic effect for up to 21 days. Viral cultures were reported as positive when definite cytopathic effect was first noted. Further identification of enteroviral isolates was done using immunofluorescent assay (IFA). The monoclonal antibody reagents were commercially prepared and purchased from Chemicon International (Temecula, CA, US). The Pan-Enterovirus Blend containing a blend of 2 mouse monoclonal antibodies was used for the preliminary identification of all enteroviruses including echovirus, coxsackie A and B virus, polio 1,2, and 3, and enterovirus 70 and 71. The Echovirus Monoclonal Antibody Blend was used for detecting common serotypes of echoviruses (serotypes 4,6,9,11,30, and 34). Enterovirus was regarded as definite etiology of aseptic meningitis when an enterovirus was isolated in CSF, and a presumptive etiology when an enterovirus was isolated from a throat and/or a rectal swab in cases of aseptic meningitis. A linear regression model was used to evaluate whether WBC counts of CSF could predict the viral isolation rate. Chi square test was also used to assess the correlation between the rate of viral isolation and the day of CSF sampling after disease onset.

Results

Clinical findings

Between January 1 and December 31, 2001, aseptic

Table 1. Clinical presentations of adult patients with aseptic meningitis (n = 38)

Symptom/sign	No. of cases (%)
Headache	38 (100)
Fever	35 (92)
Nausea and vomiting	31 (82)
Nuchal stiffness	27 (71)
Common cold	16 (42)
Myalgia	5 (13)
Abdominal pain and/or diarrhea	4 (11)
Consciousness change	4 (11)
Skin rash	1 (3)

meningitis was suspected after lumbar puncture in 38 patients who visited the emergency service of KSVGH, and 36 of these patients were admitted with an average hospital stay of 5.7 days. The age of the patients ranged from 15 to 66 years old (median, 25 years; mean, 27 years) and 33 (87%) of them were between 18 and 35 years. The male-to-female ratio was 2.16:1 (26:12 cases). The occurrence of cases peaked between June and August, accounting for 82% (31 patients) of all cases.

The clinical manifestations of the patients are shown in Table 1. Fever (35 cases, 92%), headache (38, 100%), neck stiffness (27, 71%) and nausea or vomiting (31, 82%) were the most common symptoms. The most common non-neurologic symptoms were similar to those of the common cold (42%), such as rhinorrhea or sore throat, which were also recently present among the patient's schoolmates or family members in 7 (18%) cases and might have preceded the onset of headache.

Laboratory findings

Results of analysis of the CSF in the 38 cases of aseptic meningitis are shown in Table 2. There was a wide variation in the WBC counts in CSF, ranging from 9 to 1430 cells/mm³. The mean and median WBC counts were 240 and 104 cells/mm³, respectively, and the majority of CSF WBC counts (25 cases, 65%) was less than 200 cells/mm³. Cerebrospinal fluid WBC counts between 500 and 1000 cells/mm³ were found in 5 (13%) cases and were over 1000 cells/mm³ in 2 (5%). Polymorphonuclear (PMN) cells predominated in 10 (28%) of 36 cases. The mean and median red blood cell (RBC) counts were 104 and 10 cells/mm³, respectively, with the majority (28 cases, 73%) having less than 50 cells/mm³. The average CSF protein level was 87 mg/dL (range, 40-268 mg/dL), and hypoglycorrhachia (CSF/serum glucose ratio <0.5) was found in 9 (24%) of 38 cases. Another common abnormal laboratory finding was peripheral

Table 2. Cerebrospinal fluid findings of initial lumbar puncture in adult patients with aseptic meningitis (n = 38)

CSF findings	No. of cases (%)
WBC (cells/mm³)	
0-9	2 (5)
10-99	16 (42)
100-199	7 (18)
200-299	3 (8)
300-399	2 (5)
400-499	1 (3)
500-999	5 (13)
≥1000	2 (5)
RBC (cells/mm³)	
0-49	28 (73)
50-99	3 (8)
100-199	4 (10)
200-299	1 (3)
300-999	1 (3)
≥1000	1 (3)
Protein (mg/dL)	
≤49	4 (11)
51-99	25 (66)
100-149	7 (18)
150-199	1 (3)
≥200	1 (3)
Glucose ratio (CSF/serum)	
≥0.5	29 (76)
<0.5	9 (24)

Abbreviations: WBC = white blood cell; RBC = red blood cell; CSF = cerebrospinal fluid

leukocytosis (WBC >10 000 cells/mm³), which was found in 18 (47%) of 38 cases.

Virologic findings

Cerebrospinal fluid samples, throat swabs, and/or rectal swabs were submitted for viral culture in 32 (84%) of 38 patients. The results of these cultures are shown in Table 3. Nineteen viral cultures (50%) isolated enteroviruses. Enterovirus as a definite etiology during this outbreak of aseptic meningitis was found in 17 (45%) of 38 cases, and was present as a suggestive etiology in 2 (5%) cases. Nineteen (50%) cases were

of undetermined etiology. The isolates of enteroviruses identified by IFA from culture of CSF in 17 cases were echovirus type 30 in 13 (76%) cases, echovirus type 6 in 3 (18%), and undetermined type in 1 (6%). The enteroviral isolation rates from CSF samples, throat swabs, and rectal swabs were 55% (17/31), 38% (3/8), and 60% (3/5), respectively.

Aseptic meningitis among adults in 2000

Only 5 cases of aseptic meningitis were diagnosed at KSVGH in 2000, a significantly lower prevalence compared with that in 2001. The male-to-female ratio in 2000 was 1:1.5, the average age was older (mean, 49 years; range, 33-60 years) and all cases occurred between August and October. Only one case had positive virus culture of the CSF from which echovirus of undetermined serotype was isolated.

Discussion

The 38 cases of aseptic meningitis identified among adults in 2001 at KSVGH suggested the occurrence of an outbreak, since only 5 cases were noted in 2000. Previous outbreaks of aseptic meningitis caused by enteroviruses usually occurred between summer and early autumn [1-3,6,8], as did this epidemic in 2001, which had a peak incidence between June and August. Most previously reported outbreaks of aseptic meningitis were among young infants and children [1-4,6,9,10], while outbreaks involving mainly adults have rarely been reported. In Taiwan, 2 previous outbreaks in infants and young children were reported, one in Kaohsiung in 1988 [3], and the other in Taipei in 1993 [2,4]. The male-to-female ratio (range, 1.7-2 to 1) and prevalence of echovirus type 30 as the predominant etiology were similar to the findings in this report.

The clinical signs and symptoms of enteroviral meningitis in young children are mostly nonspecific, with fever as the most common symptom, followed by irritability, lethargy, anorexia, gastrointestinal or respiratory symptoms, and rash. In older children or

Table 3. Result of viral isolation in 32 adult cases of aseptic meningitis

Specimen	No. of isolates			Isolation rate (%)
	Echovirus type 30 ^a	Echovirus type 6	Enterovirus undetermined type	
CSF (n = 31)	13	3	1	55
Throat swab (n = 8)	3	0	0	38
Rectal swab (n = 5)	3	0	0	60

Abbreviation: CSF = cerebrospinal fluid

^a2 cases had echovirus type 30 recovered from CSF, throat, and rectal swab.

Note: 6 cases had no culture; 5 had culture of CSF, throat, and rectal swab; 2 had both culture of CSF and throat swab; 1 had culture of throat swab only.

adults, the symptoms of the CNS such as headache and neck stiffness are more common [1,6], and these were also observed in this series.

In this outbreak, there were 8 cases with CSF WBC counts greater than 490×10^6 cells/L, 10 cases with a predominance of PMN cells in CSF, and 9 cases with hypoglycorrhachia. These findings suggest that it may be challenging for physicians to distinguish between viral and bacterial meningitis, especially in certain patient groups, and that special caution is needed in the differential diagnosis of groups including infants less than 1 year of age, the elderly, and immunocompromised patients [6]. In 1989, Spanos *et al* [11] proposed a logistic multiple regression model using CSF-blood glucose ratio, total PMN counts in CSF, age, and month of onset to predict whether a patient has bacterial or viral infection. Using this method, we found that the probability of bacterial meningitis was less than 0.05 in 36 (95%) of 38 cases and was 0.4 and 0.6, respectively, in the other 2 cases. Six cases received another repeated lumbar puncture, mostly within 1 day, due to predominance of PMN cells and/or hypoglycorrhachia in the CSF, and a shift towards lymphocytic predominance, a phenomenon that could be observed in viral meningitis [6,12,13], was noted in 5 cases. Several other laboratory tests of CSF useful for differentiating viral meningitis from other diseases include Limulus lysate assay, lactate, LDH, oligoclonal bands, viral specific immunoglobulin M, sialic acid, C-reactive protein, and polymerase chain reaction (PCR), but these tests have not yet become standard methods of differential diagnosis for various reasons [6,7,10].

The enteroviruses cause most acute viral infection of the CNS (35%-83%), with mumps being the second most common cause (1%-40%) [1]. Chonmaitree *et al* [9] reviewed all 111 cases of aseptic meningitis between 1979 and 1980 in patients aged between 12 days to 63 years. They found that patients with enteroviral meningitis tended to be young (all were younger than 16 years and 80% were younger than 6 months), and the predominant types of enteroviruses were echovirus 7 and coxsackievirus B3. In contrast, all patients in this study were older than 15 years and the viral isolation rate from the CSF was higher (55% vs 41%). Echovirus serotype 30 has been previously reported to affect the older age groups [10,14]. During the same period of time in 2001, the pediatric department of KSVGH submitted 288 CSF specimens for viral culture, and 72 (25%) cases yielded positive results. The serotype was echovirus type 30 in 47 (65%) cases, type 6 in 5 (7%), coxsackievirus B5 in 5 (7%), A16 in 3 (4%), A9 in 1

(2%), and undetermined enteroviruses in 11 (15%).

It has been reported that the enteroviral isolation rate from the CSF is directly proportional to the WBC counts of CSF [2,3,15]. In this study, WBC count in the CSF was not a significant indicator of positive isolation. Furthermore, we found that there was a correlation between viral isolation rate and day of CSF sampling after disease onset that was defined as the onset of headache. Virus could be isolated from 85% of the specimens (11 of 13 cases) obtained on the 1st day after disease onset, and 50% (3 of 6 cases) on the 2nd and 3rd day. Cultures were negative in specimens obtained beyond the 3rd day after the onset of headache. We also found that the rate of viral isolation was higher (13 of 15 cases, 87%) when the percentage of PMN cells in the CSF was between 10% and 70%. However, some limitations should be considered in the interpretation of our results for the evaluation of the isolation rate from our data, including differences in the quantity of CSF submitted for culture, the duration of specimen transportation to the laboratory, and the lack of specimens for culture in some cases.

Interestingly, among all of the cases we reviewed between 2000 and 2001, we found 1 case suggestive of aseptic meningitis initially that turned out to be Sjögren syndrome with CNS involvement after 3 months' follow-up. Another case with a diagnosis of Sjögren syndrome was found to have enterovirus isolated from the CSF. Two cases presented with the characteristics of Guillain-Barré syndrome or chronic encephalitis, with the absence of CSF pleocytosis, eventually had enterovirus isolated from the CSF. The diagnosis of enteroviral meningitis is especially difficult when CSF pleocytosis is absent, a phenomenon that has been reported to occur in 3% to 40% of patients [1]. Therefore, when clinical manifestations are suggestive of aseptic meningitis, cultures of stool, throat swab, and CSF for virus should be done to determine a definite etiology even in the absence of CSF pleocytosis [1,3,8].

Detection of viral ribonucleic acid by PCR has been successfully used for the diagnosis of enteroviral meningitis using specimens of CSF by Rotbart *et al* [16] since 1990 and specimens of stool by Abebe *et al* [17] since 1991. These alternative diagnostic methods hold great promise in increasing the diagnostic yield of aseptic meningitis [18] since there are many limitations in viral isolation.

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