Viral Loads of Cerebrospinal Fluid in Infants With Enterovirus Meningitis

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For a better understanding of the role of the viral load, free radicals, and cytokines in viral meningitis, we surveyed cerebrospinal fluid (CSF) obtained from patients below 1 year of age who showed positive for enterovirus. In their first examinations interleukin (IL)-6 and free radicals increased whereas pleocytosis was rarely

observed. IL-6 decreased within the short period. Viral loads and free radicals increased simultaneously. IL-6 and free radicals of CSF are helpful for diagnosis and treatment of viral meningitis at an early stage. *J. Clin. Lab. Anal.* 22:216–219, 2008. © 2008 Wiley-Liss, Inc.

Key words: viral loads; free radicals; IL-6; aseptic meningitis; meningoencephalitis

INTRODUCTION

Enteroviruses, which are a genus in the family of Picornaviridae, are known as a common pathogen of aseptic meningitis. The frequency is assumed to be 70–90% of the pathogenesis of aseptic meningitis. The severity of enteroviral infections of the central nervous system is quite variable. In Taiwan, there was a pandemic of encephalomyelitis accompanied with hand-foot-and-mouth disease with a high mortality rate in 1998 (1). We developed a highly sensitive quantitative assay and reported the association between the clinical symptoms and data of cerebrospinal fluid (CSF). However, the significance of viral loads in CSF has not fully been investigated, especially in infants below 1 year of age. Free radicals and several cytokines have also been reported to correlate with the severity of viral encephalopathy (2,3). For a better understanding of the role of the viral load, free radicals, and cytokines in viral meningitis, we surveyed those levels in CSF obtained from patients below 1 year of age.

MATERIALS AND METHODS

Subjects

We subjected 21 samples of CSF obtained from patients aged 13 days to 11 months. We classified the patients into two categories of mild (only mild symptoms:

fever, stiffness of neck, and vomiting) and moderate or severe (meningoencephalitis: convulsion, unconsciousness, and/or sequela after infection). Lumbar puncture was performed soon after admission (10 cases <24 hr, eight cases <48 hr, one case <72 hr, two cases 4 and 5 days after the appearance of fever). All samples showed positive for enterovirus by using reverse transcriptase-polymerase chain reaction (RT-PCR) (4). We assayed the quantitative assay, which was done at least twice and the mean of copy numbers was calculated. The quantitative assay was performed by using the primers and probes that were made according to the sequence data of the PCR products. The fluorescence was measured by LightCycler (Roche Diagnostics GmbH, Mannheim, Germany), which we reported previously (5).

Serum levels of interleukin (IL)-6 were assayed by chemiluminescence enzyme immunoassay (CLEIA) using Lumipulse f (Fujirebio Diagnostics, Inc., Tokyo, Japan). Nitrite/nitrate (NO_x: NO₂ and NO₃) were measured by a high-performance liquid chromatography.

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Fig. 1. The viral load (copies/ μ L) in CSFs.

graphy (HPLC)-ultraviolet (UV) system (ENO-10; EICOM, Tokyo, Japan), which we reported previously (6). The oxidative status in the CSF was studied by measuring hydroperoxide concentration according to a method automated by the D-ROM test (Diacron, Grosseto, Italy) (7,8). The serum levels of the D-ROMs test range between 250 and 300 U.CARR. (Carratelli units), where 1 U.CARR corresponds to 0.8 mg/L H_2O_2 . The CSF levels of the D-ROM in the control showed below 10 U.CARR (2).

CSF pleocytosis was defined as the presence of ≥ 35 , ≥ 21 , and ≥ 15 white blood cells (WBC)/ μ L of CSF during the first, second, and third month of life, respectively.

RESULTS

The viral loads are shown (range, 241–22,468; median, 1,569 copies/ μ L) in CSF according to the age (months) of patients, and that most patients with a high number of copy in CSF were around 1 month and 11 months old (Fig. 1). There were no significant differences at their first examination between mild and moderate to severe groups. Only four out of 21 cases showed pleocytosis in CSF (range, 0–1,536; median, 8/ μ L), and were examined within 48 hr after the appearance of fever. D-ROM in CSF was high in seven cases out of eight (39 ± 24.1 U.CARR). The levels of IL-6 were high in eight out of nine (166.0 ± 333.5 pg/mL). All cases showed high levels of IL-6 and/or D-ROM in CSF in their initial examination.

Case 1 (Day 21, Female)

Case 1 was admitted to our hospital because of vomiting and a fever of 39.8°C (Fig. 2A). On admission, her heart rate, breathing rate, and blood pressure were 172/min, 43/min, and 92 mmHg, respectively. Her physical examination was normal except for mild icterus and a rather enlarged liver. WBC count was 9,300/ μ L (neutrophils 55.3%, lymphocytes 38.9%) and C-reactive protein (CRP) was 0.6 mg/dL. Sepsis workups were all negative. CSF findings were as follows: cell count was 21/ μ L, protein was 56 mg/dL, and glucose was 59 mg/dL. The temperature fell to normal on the next day. On the fourth day after admission, the patient became

febrile again at 40°C . The CSF findings showed pleocytosis (cell count was 704/ μ L). The patient continued to run a high fever for 4 days. She started a treatment of γ -globulin intravenously. After the treatment her temperature gradually normalized. She was discharged without complication on day 21 after admission, which is shown in Fig. 2A. Coxsackie B5 was isolated from her CSF later. The levels of D-ROM, NOx, and protein showed highest at day 6. The cell counts increased at day 4 and decreased gradually. IL-6 showed highest at day 4. On the other hand NOx and protein concentration increased later.

Case 2 (2 Months of Age, Male)

Case 2 was admitted to our hospital because of unconsciousness accompanied with hand-foot-and-mouth disease (Fig. 2B). His CSF findings were as follows: cell count 0/ μ L; protein 59 mg/dL, and high IL-6 47.2 pg/mL. Four days later his second CSF analysis showed pleocytosis. The viral load and protein increased compared with the first data. However IL-6 decreased to 10.5 pg/mL. His general condition was good and he was discharged the next day.

DISCUSSION

We reported previously that the viral loads in patients under 4 years old were higher than those in patients over 4 years old (5). Patients around 1 month old who have antibodies against enterovirus and 11 months old had a high number of copy in CSF. Therefore high copy number might be caused by their weak innate immune systems (lectin and others).

Only four out of 21 cases showed pleocytosis in CSF at the first examination. CSF cell count, which is used to diagnose, was inefficient. From our results IL-6 might be a useful disease marker to diagnose enterovirus-related central nervous system (CNS) infection because of its simplicity and rapidity.

There was no significant correlation between the viral load and severity in CSF in the first examination after admission. From the fluctuation studies, IL-6 decreased within a short period. However, viral loads and free-radicals increased simultaneously. NO, a simple free radical gas, has been implicated in the pathology of many inflammatory diseases, including arthritis, myocarditis, colitis, and nephritis. Under a high concentration of NO, their reactive nitrogen species induce cell toxicity by emitting nitrosating deoxyribonucleic acid (DNA), tyrosine residues, and others (9). In experimental models of meningitis induced by intracisternally injected tumor necrosis factor (TNF) by Pfister et al. (10), NO mediates the reduction in oxygen uptake, then decreases in the CSF-blood glucose ratio, and

Fig. 2. A: The course and laboratory findings in CSF of case 1. B: The course and laboratory findings in CSF of case 2.

then intracranial pressure and lactate levels increase. Yamanaka et al. (2) reported that the CSF d-ROM levels could be valid predictive biomarkers for the severity in patients with influenza-associated encephalopathy. Therefore more consecutive CSF analysis is needed in severe enteroviral meningitis.

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