

Cytokine Response Differences in Enterovirus 71 and Other Enterovirus-caused Hand Foot and Mouth Disease Complicated with Aseptic Meningitis

CF WANG, CY CAI, SX LI, JF ZHENG, B YE, ZM CHEN

Abstract

Objectives: In order to identify EV71 meningitis-specific cytokine alteration in plasma and cerebrospinal fluid (CSF) that differs from other enterovirus (OEV)-infected ones, we compared data obtained from HFMD patients with EV71 meningitis or OEV meningitis. **Methods:** We elucidated the cytokine network based on the cytokine (IL-4, IFN- γ , IL-17A, TGF- β 1) profiles both in plasma and CSF from hand foot and mouth disease (HFMD), cases with aseptic meningitis (AM) due to EV71 (n=37) or OEV (n=26) infection using enzyme-linked immunosorbent assay. Plasma from healthy control (n=20) or CSF from febrile convulsion subjects (n=20) was run as control. Aetiological diagnosis was based on the detection of enteroviral RNA in the throat swab by real-time PCR. **Results:** Plasma IFN- γ levels were much higher in HFMD patients than controls, but no difference was shown between EV71 and OEV-infected ones. Meanwhile, plasma IL-17A levels in OEV-infected cases were significantly lower than EV71-infected subjects without differing from healthy controls. Furthermore, CSF IL-17A levels were much higher in EV71-infected groups than OEV-infected ones or subjects with febrile convulsion. In addition, no significant correlation was found between plasma cytokine levels and CSF corresponding cytokine levels. Similarly, there was no remarkable relationship between CSF cytokine levels and CSF leukocytes counts. **Conclusion:** EV71 cause different immune responses from OEV. IL-17A in the central nervous system, as well as in blood, appeared to be involved in the pathogenesis of EV71 AM.

Key words

Aseptic meningitis; Enterovirus; Hand foot and mouth disease; IFN- γ ; IL-17A

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Introduction

Hand foot and mouth disease (HFMD), is a paediatric communicable disease that mainly affects children younger than 5 years old worldwide.¹ It can be caused by more than 20 different enterovirus (EV), while Enterovirus 71 (EV71) and Coxsackie A16 (CA16) have been identified as the dominant causative agent.² In most instances, HFMD is a mild and self-limited disease with good prognosis, but some cases develop severe or life-threatening clinical manifestation presenting with aseptic meningitis (AM), poliomyelitis acute flaccid paralysis, brainstem encephalitis (BE), myocarditis, autonomic nervous system dysregulation (ANS) or pulmonary oedema (PE).

The most common clinical presentation of EV disease catches the attention of clinicians is AM and EV account for more than 80% of the known aetiology of AM.³ Previous studies indicated that pro-inflammatory cytokines (interleukine [IL]-1 β , tumour necrosis factor [TNF]- α , IL-6, and interferon [IFN]- γ) and anti-inflammatory cytokines (IL-10, IL-4, and transforming growth factor (TGF)- β 1) are involved in the modulation of immune and inflammatory processes in AM.⁴ Wang et al demonstrated an association between the extent of cytokines (IL-8, IL-1 β , IL-6, IFN- γ) response in the central nervous system (CNS) and varying severity of HFMD.⁵ Those results suggested the possibility of immune-mediated mechanism of AM.

Although the clinical characteristics and laboratory findings in EV AM and HFMD have been well studied, key laboratory findings as potential indicators of HFMD are not well known, and in particular, the local immune response has not been fully elucidated yet. There have been several researches on cerebrospinal fluid (CSF) cytokine profile in aseptic meningitis,^{5,6} whereas few studies were performed to compare the cytokine profiles among the different pathogen-associated HFMD in China. Meanwhile, the majority of studies dealing with this topic emphasized on patients with more severe forms of CNS inflammation caused by EVs, particularly those with EV71 BE.^{5,7} Additionally, the mechanisms underlying EV71 infection were still unclear and most likely multi-factorial, in which both immunological and non-immunological responses, as well as genetic susceptibility play a role.⁸

Therefore, the purpose of current study was to study the cytokines (IL-4, IFN- γ , IL-17A, TGF- β 1) in the plasma and CSF of HFMD children with AM and compare the observed pattern of expression between EV71-infected and other enterovirus (OEV)-infected cases. The results of this study were expected to determine whether abnormal

expression of cytokines in the plasma and CSF contributes to the induction of local cellular immune response in HFMD patients with AM and identify EV71 meningitis-specific cytokine alternations in CSF compared with OEV-infected patients.

Methods

Definitions and Samples

This prospective, cross-sectional study was carried out at Children's Hospital, Zhejiang University School of Medicine and Hangzhou Children's Hospital between April 2013 and September 2013 during the epidemic period of HFMD. The diagnosis of HFMD was based on clinical signs and symptoms (including fever, oral ulcers and papulovesicular / papular rash on the hands, feet, knees, and buttocks). AM was diagnosed on the basis of clinical features (including headaches, irritability, myoclonic jerk), CSF pleocytosis (≥ 10 cells/mm³), negative Gram stain and bacteriological culture. The EV aetiology was confirmed by real-time polymerase chain reaction (PCR). CSF samples were collected from the HFMD patients with meningitis. Children with febrile convulsion and clinical suspicion of CNS infection at the admission to the hospital, but in whom the initial diagnosis was ruled out based on negative CSF cytology, bacteriological analysis, as well as the exclusion of EV infection by real-time PCR were run as CSF control. These patients with febrile convulsion were later proved to have an extraneural site of infection (n=20). Peripheral blood were collected from HFMD patients with meningitis mentioned above, and healthy children without any infection and immunological disease (n=20). All samples (blood, CSF) were collected during the acute phase of the illness. The Ethic Committee of Children's Hospital, Zhejiang University School of Medicine and The Hangzhou Children's Hospital approved this study protocol. Informed consent was obtained from the children's parents or guardians. The CSF white blood cell (WBC) count, glucose, protein and chloride levels were determined by standard laboratory procedures.

Cytokine Detection

The supernatant of CSF was stored at -70°C until cytokine measurements after centrifuged (2000 g for 10 minutes). Plasma was separated from blood samples by centrifugation (2000 g for 10 minutes) at 4°C and stored at -70°C until cytokine analysis. Concentration of cytokines (IL-4, IFN- γ , IL-17A, TGF- β 1) was determined by enzyme-

linked immunosorbent assay (ELISA) kits (Quantikine, R&D Systems, USA) following the manufacturer's instructions. Samples were measured in duplicate and the concentrations were calculated from the standard curve of duplicate standards. The assay sensitivities for IFN- γ , IL-4, IL-17A, TGF- β 1 is 0.2 pg/ml, 0.2 pg/ml, 0.2 pg/ml and 4.6 pg/ml, respectively. When cytokines were not detectable, the minimal detectable level was used in the calculations.

Aetiological Diagnostics

Throat swab specimens were collected from each HFMD child and controls enrolled in this study. The enterovirus nucleic acid detection kit (Da An Gene Co., Ltd. of Sun Yat-sen University, China) was used for detection of EV71 and panenterovirus based on the TaqMan PCR Technology. Viral RNA was isolated with viral RNA purification kits (Hangzhou Haofeng Biotechnology Co., Ltd. Hangzhou, China). RT-PCR was performed using the one step primescript RT-PCR kit (TaKaRa, Dalian, China) according the manufacturer's instruction. The RT-PCR thermal profile consisted of 25 minutes at 40°C, 3 minutes at 94°C, and then followed by 40 cycles of 15s at 93°C and 45s at 55°C. Reverse transcription, amplification, detection, and data analysis were performed with Applied Biosystems 7500 real-time PCR system (Applied Biosystems, Inc., CA, USA).

Statistics

Kolmogorov-Smirnov test was used to test the normality of quantitative data. Continuous variables that were not distributed normally were expressed as median and interquartile range (25%-75%) and analysed by nonparametric Kruskal-Wallis test or Mann-Whitney U-test, while variables normally distributed variables were presented as means \pm SD and analysed by one way analysis of variance (ANOVA). χ^2 test was used for categorical data. A Spearman's rank or Pearson's correlation coefficient test was employed to examine the strength and pattern of association for continuous variables. *P* values below 0.05 were considered statistically significant. All analyses were performed by SPSS version 17.0 (SPSS Inc., Chicago, IL, USA).

Result

Demography and CSF Analysis

Based on the real time PCR results, the HFMD patients with AM investigated consisted of 37 EV71-infected subjects and 26 OEV (panenterovirus, exclude EV71)-infected ones. Then the demographical data and CSF finding

for HFMD patients with EV71 meningitis or OEV meningitis are shown in Table 1. EV71-infected patients had longer hospital stays than OEV-infected ones ($p=0.001$). The white blood cell (WBC) count of peripheral blood was higher in EV71-infected group compared with OEV-infected group ($p=0.004$), so was CSF WBC number ($p=0.002$). There were no differences between EV71 meningitis and OEV meningitis patients with regard to C-reactive protein (CRP) levels and CSF total protein levels ($p=0.553$, $p=0.246$). No significant differences were found in other baseline characteristics between these two groups, either. None of these HFMD patients developed into fatal cases including BE, PE, death.

Cytokine Concentrations in Plasma and CSF

Plasma IFN- γ concentrations in HFMD children with AM caused by OEV or EV71 were markedly higher than control group ($p=0.015$, $p<0.001$, respectively), but no difference was found between OEV and EV71-infected ones ($p=0.386$). Plasma IL-17A levels were significantly lower in OEV-infected subjects than in subjects with EV71-infection ($p=0.001$), but it didn't differ from healthy controls significantly ($p=0.252$). Comparison of plasma IL-4, TGF- β 1 levels among these three groups revealed no significance (Table 2).

CSF IL-17A showed higher concentrations in EV71-infected cases when compared to OEV-infected cases or patients with febrile convulsion ($p=0.003$, $p<0.001$), but no statistically significance showed between the latter two groups ($p=0.261$). It indicated no difference in CSF IL-4, IFN- γ , TGF- β 1 levels among these three groups (Table 2). To further evaluate whether the increased plasma or CSF IL-17A levels had any potential clinical significance as a possible biomarker for discriminating EV71 meningitis from OEV cases, a receiver operating characteristic (ROC) curve was constructed by plotting sensitivity vs. specificity (Figure 1). When the cut-off values for plasma IL-17A and CSF IL-17A were set at 2.81 pg/ml and 17.71 pg/ml, respectively, the sensitivity and specificity in differentiating EV71 cases from OEV cases were 81.1% and 73.1%, and 51.4% and 88.5%, respectively.

Correlation Between the CSF Levels of Cytokine and the Plasma Levels of Corresponding Cytokine, Mean Ratio of CSF to Plasma Cytokine Concentration According to EV71 or OEV Infection

There was no apparent correlation between cytokine levels in CSF and corresponding cytokine levels in plasma (Table 3). The data shown in Table 4 indicated the

Table 1 Comparison of demography and cerebrospinal fluid findings for HFMD patients with AM caused by EV71 or OEV

| Characteristic | EV71 (n=37) | OEV (n=26) control(n=20) | Healthy convulsion (n=20) | Febrile | <i>p</i> |
|-------------------------------|---------------------|-----------------------------|------------------------------|---------------------|----------|
| Ages (years) | 2.4±1.1 | 3.0±1.5 | 2.5±1.3 | 2.3±1.4 | 0.124 |
| Gender (male/female) | 21/16 | 18/8 | 10/10 | 10/10 | 0.392 |
| Hospital stays (days) | 9.0±1.8* | 7.6±1.9 | – | 5.0±1.2 | <0.001 |
| Blood routine | | | | | |
| WBC | 14.7 (12.7-18.2)* | 12.0 (9.5-14.4) | 8.7 (6.4-9.2) | – | <0.001 |
| Neutrophils (%) | 62.4±14.6 | 59.8±18.2 | 55.3±17.0 | – | 0.294 |
| Lymphocytes (%) | 29.5±13.6 | 30.0±14.0 | 37.2±14.8 | – | 0.123 |
| Hb | 120.0±9.9 | 120.5±12.3 | 121.7±7.6 | – | 0.831 |
| Plt | 293.0±60.3 | 295.0±62.3 | 293.8±53.7 | – | 0.992 |
| CRP | 13.0 (3.5-35.3) | 4.7 (1.8-26.0) | 0.0 (0.0-0.5) | – | <0.001 |
| Glucose (mmol/L) | 5.5±1.0 | 5.4±1.2 | 5.5±1.1 | – | 0.880 |
| CSF WBC (×10 ⁶ /L) | 70.0 (42.5-181.5)* | 39.0 (15.8-72.5) | – | 5.0 (2.0-5.8) | <0.001 |
| Neutrophils (%) | 52.2±26.4 | 40.0±28.5 | – | – | 0.101 |
| Lymphocytes (%) | 41.4±25.6 | 53.1±28.5 | – | – | 0.110 |
| Monocytes (%) | 6.2±3.7 | 6.2±2.9 | – | – | 0.96 |
| Total protein (g/L) | 326.0 (264.5-404.0) | 287.0 (229.5-384.5) | – | 184.0 (137.0-241.0) | <0.001 |
| Glucose (mmol/L) | 3.9±0.8 | 3.9±0.7 | – | 3.8±0.9 | 0.909 |
| Chloride (mmol/L) | 124.4±3.3 | 124.4±2.6 | – | 125.2±2.3 | 0.598 |

**p*<0.05: EV71-infected cases vs OEV-infected cases

WBC: white blood cell; Hb: haemoglobin; Plt: platelet; CRP: C-reactive protein.

Table 2 Cytokine in plasma and cerebrospinal fluid in HFMD children with AM caused by EV71 or OEV

| Cytokine | Plasma | | | | CSF | | | |
|-----------------------------|------------------------------|------------------------------|---------------------------|----------|--------------------------------|----------------|---------------------------------|----------|
| | EV71 (n=37) | OEV (n=26) | Healthy control (n=20) | <i>p</i> | EV71 (n=37) | OEV (n=26) | Febrile convulsion (n=20) | <i>p</i> |
| IL-4 (pg/ml) | 0.9 (0.4-12.2) | 1.4 (0.4-10.3) | 0.7 (0.3-1.4) | 0.176 | 0.8 (0.2-1.0) | 0.8 (0.2-0.9) | 0.9 (0.2-1.0) | 0.503 |
| IFN-γ (pg/ml) | 47.4 (5.9-96.6) ^b | 13.3 (2.0-89.7) ^a | 3.2 (0.2-12.5) | 0.002 | 9.4 (3.2-22.5) | 6.5 (3.1-22.8) | 4.1 (0.3-9.4) | 0.156 |
| IL-17A (pg/ml) | 4.8 (3.1-8.7) ^c | 1.8 (0.2-3.5) | 3.8 (0.2-11.0) | 0.011 | 18.3 (5.5-30.4) ^{c,d} | 6.5 (0.2-14.2) | 2.2 (0.6-5.4) | <0.001 |
| TGF-β1 (ng/ml*, pg/ml**) | 36.4±18.9 | 42.3±18.2 | 39.6±12.4 | 0.402 | 7.6 (7.6-26.2) | 7.7 (7.6-10.8) | 7.6 (7.6-7.6) | 0.494 |

Data are medians (interquartile range) or means ± SD and calculated with Kruskal-Wallis test or AVONA accordingly.

^a*p*<0.05, for OEV infection cases vs Healthy control group^b*p*<0.05, for EV71 infection cases vs Healthy control group^c*p*<0.05, for EV71 infection cases vs OEV infection cases^d*p*<0.05, for EV71 infection cases vs Febrile convulsion cases

ng/ml* is the unit for plasma TGF-β1, while pg/ml** is for CSF TGF-β1.

Table 3 Correlation between the cytokine levels in CSF and corresponding cytokine levels in plasma according to EV71 or OEV infection

| Cytokine | EV71 (n=37) | | | | OEV (n=26) | | | |
|----------------|-------------|--------|--------|--------|------------|--------|-------|--------|
| | IL-4 | IFN-γ* | IL-17* | TGF-β1 | IL-4 | IFN-γ | IL-17 | TGF-β1 |
| <i>r</i> | -0.093 | -0.085 | -0.295 | 0.065 | 0.303 | -0.037 | 0.255 | 0.042 |
| <i>p</i> value | 0.585 | 0.618 | 0.077 | 0.703 | 0.133 | 0.856 | 0.209 | 0.840 |

Data are calculated with spearman correlation or pearson correlation (*).

concentration of IFN- γ and IL-17 in CSF were much higher than those in plasma, while IL-4 and TGF- β 1 were just the opposite both in EV71 and OEV-infected patients. TGF- β 1 was the only cytokine among those cytokines which the CSF levels never exceed the plasma values (Table 4). Analysing the possible contribution of pleocytosis on cytokine concentration in the CSF, no strong correlation was found for these four cytokines in EV71-infected cases or OEV-infected ones (Table 5).

Discussion

Here, we showed elevated IFN- γ profiles in plasma and increased IL-17A profiles in CSF in HFMD patients with AM. Normally, the cellular expression of cytokines in the CNS is under strict regulation as CNS is regarded as an immunological privileged site. However, in some pathological state, abnormal expression cytokine may contribute to inflammation and immunologic events occurring in the CNS.^{4,7,9,10} It was confirmed that the complex network of cytokines/chemokines was interfered by *Streptococcus agalactiae* with the breakdown of the Blood-brain barrier.⁹ H5N1 infection could induce a long-lasting inflammatory response in brain and play a contributing factor in the development of pathologies in neurodegenerative disorders.¹¹ Prior studies verified that EV71 may induce cytokines released into the systemic

compartment and overexpress chemokines in CNS to elicit of the immune response.^{7,10} Taken these studies into consideration, our results also indicate that cytokine disorder was involved in the pathogenesis of HFMD with AM.

In present study, we found EV71 meningitis led to longer hospitalisation period compared to OEV meningitis. Similarly, a case-control study showed that EV71 infection was one of the risk factors for severe HFMD.¹² EV71 infection attracted worldwide attention as it contributed more for severe and fatal cases.^{13,14} These studies revealed that the severity of EV71 infection surpassed OEV cases. EV71-associated HFMD should be monitored closely in order to give early warning so as to get prompt treatment. Generally, in viral meningitis, the typical profile is normally a lymphocytic pleocytosis, a normal or slightly elevated protein concentration, and a normal or mildly elevated

Table 4 Mean ratio of CSF to plasma cytokine concentrations in HFMD patients with AM caused by EV71 or OEV

| Cytokine | EV71 | OEV |
|------------------------------------|-----------------|----------------|
| IL-4 | 1.7 \pm 5.0 | 0.7 \pm 1.3 |
| IFN- γ | 11.0 \pm 54.4 | 9.7 \pm 26.6 |
| IL-17 | 9.9 \pm 16.3 | 9.1 \pm 17.0 |
| TGF- β 1($\times 10^{-4}$) | 12.4 \pm 22.1 | 3.8 \pm 5.2 |

Data are means \pm SD.

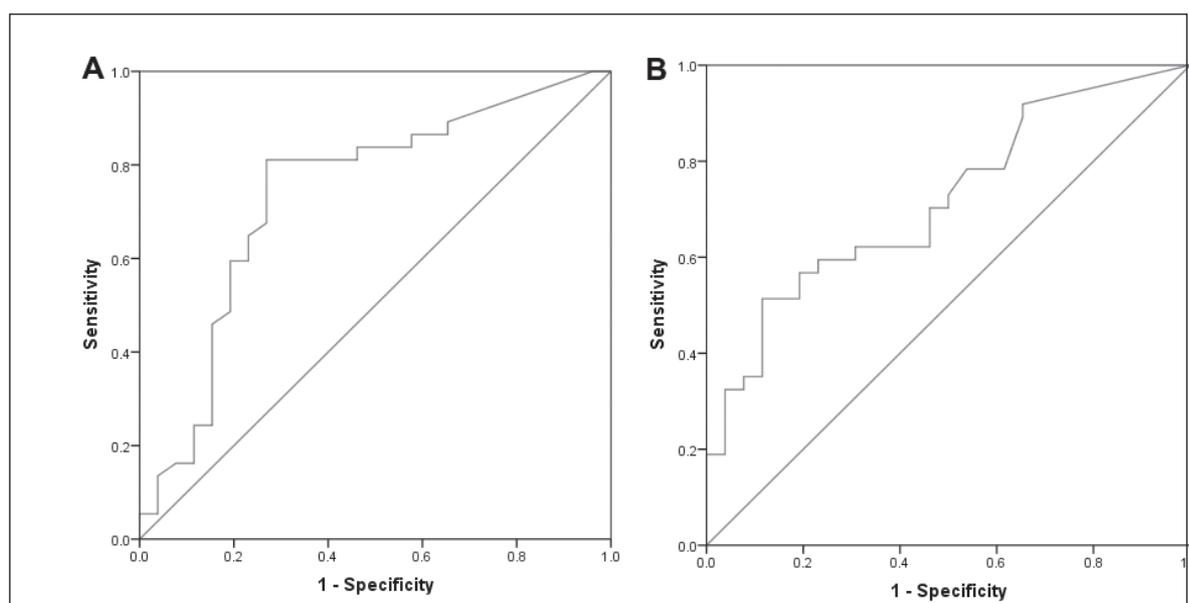


Figure 1 ROC for distinguishing EV71 meningitis from OEV cases. (A) plasma IL-17A; (B) CSF IL-17A.

opening pressure. In our study, we noted patients with EV71 infection had significantly higher WBC but relative lower lymphocyte frequencies in CSF than those with OEV infection, which was in agreement with result of previous study.¹⁵ Based on the results, we suspected that meningitis caused by different virus has various CSF characteristics. It is indicated that lymphocytes depletion (including lower circulation CD4+ T cells, CD8+ T cells, and natural killer cells) is associated with HFMD patients with PE caused by EV71.¹⁰ Human immunodeficiency virus (HIV) induced the progressive loss of CD4+ T lymphocytes.¹⁶ Chen et al. found that EV71 inducing FasL expression, contributed to T cell apoptosis.¹⁷ Other studies also found EV71 was able to infect, active, and induce apoptosis of endothelial cells and neuronal apoptosis.¹⁸⁻²⁰ Taken these together, we suggest decreased lymphocyte percentage might be a sign of EV71 infection. This could be used as a functional marker for EV71-related cases and may enrich the definition of HFMD caused by different pathogen.

IL-17A, as a member of pro-inflammatory mediators, has been linked to many immune and auto-immune related diseases. In our study, we found IL-17A profiles in EV71 meningitis differed from those in OEV meningitis, indicating that EV71 cause different immune responses from OEV. Although effector function are not fully understood, it is appreciated that IL-17 is capable of inducing a specific pro-inflammatory immune response required for clearance of bacteria, virus, and fungi, possibly through induction of a neutrophil response.²¹ It was found that the pro-inflammatory action of Th17 cells through the induction of neutrophil-recruiting chemokines (CXCL1, CXCL2, CXCL8) by IL-17.²² IL-17A signal induces granulocyte colony-stimulating factor and stem cell factor through the IL-17R and thereby expands neutrophil progenitors in the bone marrow and spleen as well as increasing mature neutrophils in the blood.^{23,24} It was also speculated that pro-inflammatory cytokines produced

attracting WBCs into CNS immediately after enterovirus infection, and then terminated by the production of anti-inflammatory cytokines after elimination of virus.²⁵ Thus, higher WBC count both in blood and CSF in present study may be the result of elevated IL-17 levels in EV71-infected cases when compared to OEV meningitis. Chen et al. also found the frequencies of Th17 cells and IL-17 levels in peripheral blood samples increased in HFMD patients caused by EV71.²⁶ These together reflected the EV71 may cause different response in the activity of IL-17A and IL-17A may play an important role in the host response to EV71 infection. Further ROC analysis revealed that both plasma IL-17A and CSF IL-17A levels could help distinguish EV71 cases from OEV cases as a diagnostic biomarker, which may guide patient treatment in clinical practice.

We didn't find any positive or negative correlation between CSF leukocyte count and cytokine expression nor between CSF cytokine and plasma corresponding cytokine. It was convinced that cells of the innate immune system including polymorphonuclear leukocytes (PMNs, neutrophils, and eosinophils), macrophages, and nature killer (NK) cells from outside the CNS (infiltrating cells), and cells within the brain parenchyma including microglia, astrocytes and neuron can produce most of the cytokines found in CSF inflammation.²⁷ Cytokines don't act separately but involved in a cascade and complicated network in immune response. Thus, it was also not surprising that levels of IFN- γ and IL-17A in CSF were remarkably exceeded plasma levels in HFMD children, as cytokines were mainly produced locally.⁷

There are several limitations in this study. First, some studies have suggested that young infants with enterovirus infections of the CNS lack of CSF pleocytosis.^{28,29} Our present study only included patients with CSF pleocytosis might have caused possible selection bias. Second, the most appropriated control group would be patients with no fever or infectious syndromes, but it is hard to reach

Table 5 Relationship between cytokine levels in CSF and CSF pleocytosis

| CSF parameters | EV71 | | | | | | | | OEV | | | | | | | |
|---------------------------|--------|-------|-----------------|-------|---------|-------|----------------|-------|--------|-------|-----------------|-------|---------|-------|----------------|-------|
| | IL-4 | | IFN- γ^* | | IL-17A* | | TGF- β 1 | | IL-4 | | IFN- γ^* | | IL-17A* | | TGF- β 1 | |
| | r | p | r | p | r | p | r | p | r | p | r | p | r | p | r | p |
| WBC (x10 ⁶ /L) | 0.083 | 0.625 | 0.203 | 0.468 | -0.127 | 0.454 | -0.017 | 0.921 | -0.179 | 0.383 | -0.108 | 0.600 | -0.108 | 0.600 | 0.257 | 0.205 |
| Neutrophils (%) | 0.161 | 0.347 | 0.035 | 0.840 | 0.022 | 0.897 | 0.284 | 0.093 | -0.086 | 0.705 | 0.061 | 0.787 | 0.423 | 0.171 | -0.043 | 0.85 |
| Lymphocytes (%) | -0.169 | 0.324 | -0.073 | 0.672 | -0.024 | 0.888 | -0.106 | 0.538 | 0.110 | 0.626 | -0.075 | 0.739 | -0.451 | 0.141 | 0.283 | 0.201 |
| Monocytes (%) | 0.05 | 0.775 | 0.285 | 0.097 | -0.011 | 0.950 | 0.256 | 0.137 | 0.235 | 0.292 | 0.044 | 0.844 | 0.082 | 0.716 | -0.017 | 0.941 |

Data are calculated with spearman correlation or pearson correlation (*).

CSF from them. Third, because it was reported that EV-PCR of CSF yielded positive results for only 31.2%,³⁰ PCR of throat swab specimens were considered for higher diagnostic yields in present study. This prevented us from observing the relationship between viral load in CSF and local IL-17A levels. Hence, future studies with animal model should be carried out to explore the clinical application of this observation whether elevated levels of IL-17A in plasma and CSF can be a biomarker distinguishing EV71 from OEV.

Conclusion

We observed enhanced IL-17A levels both in plasma and CSF in HFMD patients with EV71 meningitis as compared with OEV meningitis indicated the pathogenesis of EV71 differs from OEV and IL-17A may be involved in the pathogenesis of EV71.

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Conflict of Interest

There was no conflict of interest.

Contributions

All authors contributed to the planning and performing the experimental work, collecting samples and clinical data, interpretation of the results, and preparation of the manuscript. Zhimin Chen acts as the guarantor of this article.

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