

Efficacy of high-dose methylprednisolone pulse therapy in the treatment of enterovirus 71 encephalitis

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Abstract: To investigate the efficacy of high-dose methylprednisolone pulse therapy in the treatment of Enterovirus 71 (EV71) encephalitis. To determine whether high-dose methylprednisolone pulse therapy should be used, 80 cases of pediatric patients with EV71 encephalitis were randomly divided into steroid pulse therapy group and non-steroid pulse therapy group and their clinical information was compared using statistic analysis. There was no statistical difference in the duration of fever, duration of nervous system involvement, duration of hospital stay, blood pressure, and cure rates between the two groups ($p>0.05$). The heart rate, respiratory rate, white blood cell counts and blood glucose of the steroid pulse therapy group were significantly higher than those of the non-steroid pulse therapy group ($p<0.05$). High-dose steroid pulse therapy to treat EV71 encephalitis can't shorten the course or improve the prognosis of the disease. In contrast, it has side effects and might aggravate disease condition or interfere with disease diagnosis. Our study suggested that there is no beneficial effect to use high-dose steroid pulse therapy for the treatment of EV71 encephalitis.

Keywords: Enterovirus 71, encephalitis, glucocorticoid, clinical research.

INTRODUCTION

Enterovirus71 (EV71) is a member of the family Picornaviridae. It infects humans by the fecal-oral route and can cause fever and other mild symptoms, such as herpangina or hand-foot-and-mouth disease (HFMD). As a neurotropic pathogen, EV71 can cause aseptic meningitis, encephalitis, brainstem encephalitis, acute flaccid paralysis and other neurological diseases with various severity and prognosis. In the 1970s, the major clinical manifestations of EV71 infection in the nervous system included aseptic meningitis, encephalitis and acute flaccid paralysis for patients in the United States, Bulgaria and Hungary and the prognosis was fairly good. However, after the 1990s, in Malaysia, Taiwan and mainland China, a small number of patients with severe central nervous system involvement showed rapid progression and poor prognosis (Wang and Liu 2009).

In recent years, there have been continuous large-scale outbreaks of EV71-caused HFMD, often with death, in some of the regions in mainland China. Due to the lack of effective antiviral treatment for HFMD, many Chinese researchers (He *et al.*, 2009, Sun *et al.*, 2011) have proposed the use of high-dose methylprednisolone pulse therapy to treat EV71 infection during the nervous system involvement period. These studies suggested that high-dose methylprednisolone pulse therapy might reduce systemic inflammatory responses and prevent the progression of EV71 infection to heart and lung failure, thus effectively preventing disease progression and

reducing mortality in pediatric patients. It has been shown that the level of proinflammatory cytokines IL-1 β and IL-6 and chemokine IL-8 was significantly elevated in the plasma and cerebrospinal fluid (CSF) of children with severe EV71 infection (Lin *et al.*, 2003, Wang *et al* 2007, Wang *et al.*, 2008). The inflammatory responses triggered by cytokine over-activation might be the important factor for EV71-caused neurogenic pulmonary edema (NPE). Both glucocorticoids and immunoglobulins can inhibit inflammatory reactions by blocking the synthesis of inflammatory cytokines. In addition, glucocorticoids can induce the synthesis of anti-inflammatory cytokine, inflammatory proteins and lipocortin, and inhibit the synthesis of leukocyte inflammatory proteases. They can inhibit the recruitment of monocytes, neutrophils and macrophages to sites of inflammation, prevent phagocytosis, and induce the apoptosis of inflammatory cells (Glezer and Rivest 2004). Therefore, it is hypothesized that early application of glucocorticoid treatment can effectively reduce inflammation and improve prognosis for EV71-caused inflammation of the central nervous system.

However the use of glucocorticoids for EV71 infection remains controversial. Some researchers (Fu *et al.*, 2009, Osuchowski *et al.*, 2006) have suggested that during the early stage of EV71 infection, over-synthesis of pro-inflammatory cytokines such as TNF- α causes systemic inflammatory response syndrome (SIRS). With the progression of inflammation, anti-inflammatory cytokines such as IL-10 are generated, inducing compensatory anti-inflammatory response syndrome (CARS) or mixed

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antagonist response syndrome (MARS). When patients are presented with CARS or MARS, steroids should be used with caution. In addition, animal experiments (Shen *et al.*, 2014) have shown that glucocorticoids can reduce the number of CD4⁺ T cells, CD8⁺ T cells and CD19⁺ B cells, causing potential spread of EV71. Thus steroid treatment is ineffective for EV71 infection. Furthermore, one recent report with 134 cases has shown that early use of glucocorticoids to treat fever in outpatients increased risk of severe disease or death during the 2008 outbreak in China (Ma *et al.*, 2010). Use of glucocorticoids might also be associated with a number of deaths in Cambodia in 2012 (Seiff A 2012). Therefore, further investigation is required regarding the use of high-dose glucocorticoid therapy for severe EV71 infection. In this study, we chose children with EV71-caused HFMD and subjected them to high-dose methylprednisolone pulse therapy when encephalitis or meningoencephalitis was developed. Control group was treated without the use of steroids. The efficacy, side effects, duration of fever, length of hospital stay, vital signs, laboratory tests and other indicators of outcomes between two groups were compared and the clinical value of glucocorticoid therapy for EV71 infection was evaluated.

MATERIALS AND METHODS

Case inclusion criteria

80 cases of HFMD pediatric patients with EV71 encephalitis who were treated at the Taian City Central Hospital between March 2014 and September 2014 were included in this study. Patients were randomly divided into two groups of 40 each, including the steroid pulse therapy group and non-steroid pulse therapy group. Patients in the latter group were confirmed to have not used glucocorticoids previously. Institutional review board approval was obtained from Taian City Central Hospital for this study and informed consent was obtained from all patients or their parents.

Diagnostic criteria

Diagnostic criteria for EV 71 infection with nervous system involvement Aseptic meningitis: The clinical manifestations include irritability, poor spirit, headache, vomiting, muscle pain, fatigue, and positive meningeal irritation. There is nonbacterial change in CSF and the main cells in CSF are lymphocytes. No bacteria are found by smear detection and CSF culture is negative for bacteria.

Encephalitis: There are obvious symptoms and signs of brain parenchymal lesions, such as disturbance of consciousness, mental abnormalities, physical disabilities, seizures, and pyramidal signs. Similarly as in aseptic meningitis, there is nonbacterial change in CSF. EEG shows abnormal background activity and increase in slow wave. Brainstem encephalitis: The clinical characteristics

include one-side or two-side brainstem involvement, such as nystagmus, pupil abnormalities, myoclonus, ataxia, tremors, ataxia, cranial nerve dysfunction, tachycardia, abnormal bowel movements, vomiting, high blood pressure, difficulty swallowing, and awareness changes. CSF is normal or has mild change that is not caused by bacteria. MRI indicates brainstem lesions. Patients with clinical manifestations of both meningitis and encephalitis are identified as meningoencephalitis patients.

Flaccid paralysis: The clinical manifestations include acute limb weakness or paralysis, decrease in muscle and tendon reflexes, and bladder retention. According to the treatment programs for EV71 infection proposed previously (Lin *et al.*, 2002), pediatric patients with HFMD were divided into different clinical stages, including Stage I: Appearance of rash on hand, foot and mouth; Stage II: Nervous system involvement; Stage IIIA: Early stage of heart and lung failure; Stage IIIB: Cardiopulmonary failure and Stage IV, recovery.

Etiology detection

The diagnosis of EV71 infection was established by reverse-transcriptase polymerase chain reaction (RT-PCR) from one or more throat swabs, stool specimens, CSF, or other tissue fluids that were collected from each patient on the day of admission, and were genotyped based on the sequence analysis of VP1. Primers used are as follows:

EV71-S: 5'- GCA GCC CAA AAG AAC TTC AC -3' (VP1 2646-2664)

EV71-A: 5'- ATT TCA GCA GCT TGG AGT GC -3' (VP1 2986-2967)

The PCR condition used is as follows: 50°C for 30 min, 95°C for 15min, 94°C for 1min, 52°C for 45s, 72°C for 1 min, and 72°C for 10 min, total 40 cycles. PCR products were detected by electrophoresis on 2% agarose gel. Patients with positive EV71-specific RT-PCR products were identified as having EV71 infection.

Treatment plans

All patients received ribavirin 10 mg/kg/d and mannitol 0.5 - 1.0 g/kg, once every 4 - 8 h; and gamma globulin 1 g/kg/d, 2 g/kg. Patients were also treated for fever relief and their fluid, electrolyte and acid-base balance was maintained. Steroid pulse group received a dose of methylprednisolone at 10mg/kg/d, for three continuous days. Disease progressed in seven children, who were provided with ventilator breathing. 2 of these patients died.

Data analysis

The clinical data, including age, sex, weight, duration of fever, length of hospital stay, vital signs and laboratory tests and outcomes, were collected and analyzed for both the steroid treatment group and non-steroid treatment group. Some data were collected both at the time of admission and 48h-72h after hospitalization.

STATISTICAL ANALYSIS

Enumeration data were presented as mean \pm standard deviation ($\bar{x} \pm s$) and analyzed using SPSS 20.0 statistical software. Statistical significances were determined using either χ^2 test (categorical variable data), or t-test/t'-test (numerical variable data). $p < 0.05$ was considered statistically significant.

RESULTS

Clinical manifestations and staging results

All 80 pediatric patients (100%) at admission were presented with various degrees of nervous system involvement, including poor spirit, drowsiness, easily frightened, headache, vomiting, irritability, shaking limbs, acute limb weakness and neck stiffness. CSF examination suggested aseptic meningitis. 8 children underwent cerebrospinal CT scan and the findings were negative. 13 children underwent cerebrospinal MRI, nine of whom showed abnormal high signals of the brainstem on T2-weighted images (figs. 1-4), and the rest were normal. 29 children (36.25%) showed signs of early-stage heart and lung failure, including increase in heart rate and respiratory rate, cold sweats, skin pattern, cold extremities, elevated blood pressure, elevated blood glucose, elevated leukocytes and abnormal cardiac ejection fractions. Based on the clinical characteristics and results of laboratory examinations, we diagnosed 6 cases of viral meningitis, 72 cases of viral encephalitis (including 64 cases of brainstem encephalitis), and 2 cases of acute flaccid paralysis. The most common clinical manifestations included fever (100%), poor spirit (100%), myoclonic twitching (84%), vomiting (52%) and easily frightened (50%). Nervous system involvement and staging results for both groups were shown in table 1. The difference between the methylprednisolone treatment group and non-steroid treatment group was not statistically significant ($P > 0.05$).

Results of methylprednisolone pulse therapy

General clinical data

As shown in table 2, the difference in sex, age, body weight, temperature, duration of fever, duration of nervous system involvement and hospitalization between the methylprednisolone pulse group and non-steroid pulse group was not statistically significant ($p \geq 0.05$).

Vital signs and laboratory findings

As shown in table 3, there was no statistical difference in vital signs and laboratory findings at admission between the steroid group and non-steroid group. As shown in table 4, 48-72 h after hospitalization, the difference in SBP, DBP, CRP, Hb and PLT between the two groups was not statistically different. On the other hand, the HR (144.13 ± 15.24 : 137.43 ± 10.75 , $t = 2.950$, $p = 0.005$), RR (38.18 ± 8.05 : 35.20 ± 6.04 , $t = 2.423$, $p = 0.020$), WBC (14.87 ± 3.72 : 12.75 ± 3.32 , $t = 2.896$, $p = 0.006$) and GLU

(9.09 ± 3.87 : 7.74 ± 2.28 , $t = 2.067$, $p = 0.045$) in the steroid therapy group were significantly higher than those in the non-steroid therapy group ($p \leq 0.05$).

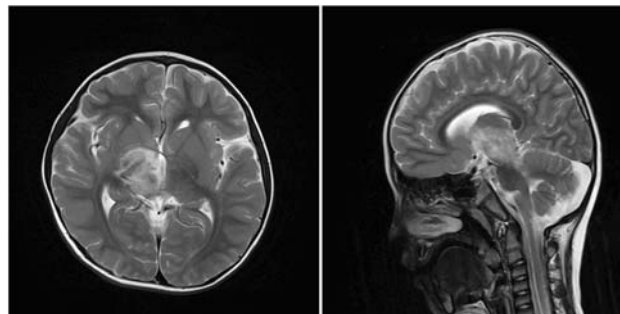


Fig. 1: An MRI study of patient 1 showed the signal shade long T2 in the right basal ganglia region, right thalamus and midbrain on T2-weighting.

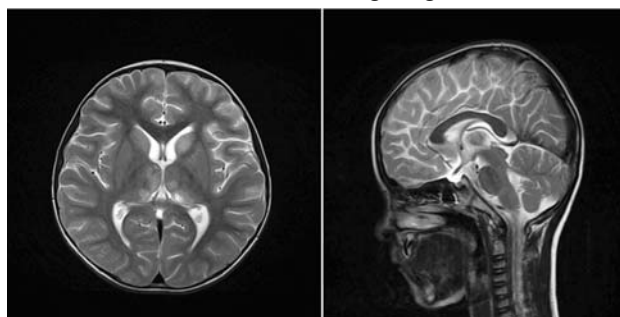


Fig. 2: An MRI study of patient 2 showed the patchy signal shade long T2 in the bilateral caudate nucleus, thalamus, hippocampus, right cerebral peduncle on T2-weighting.

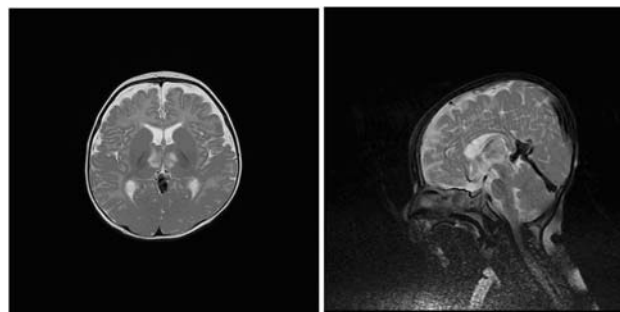


Fig. 3: An MRI study of patient 3 showed the symmetrical signal shade long T2 in the bilateral thalamus, midbrain, cerebral peduncle on T2-weighting. Significant hyperintensity on FLAIR.

Treatment effects

Children had sequelae at the time of discharge and were considered improved, including 1 case with flaccid paralysis and 1 case with epilepsy in the steroid treatment group and 1 case with flaccid paralysis, 1 case with swallowing dysfunction and 1 case with cognitive function obstacles in the non-steroid treatment group. 2 patients in the steroid pulse therapy group died. The cure rate between the two groups was not statistically significant ($p > 0.05$).

Table 1: Nervous system involvement and staging results

	Viral Meningoencephalitis	Viral Encephalitis	Acute Flaccid paralysis	Stage II	Stage IIIA
Methylprednisolone pulse group (cases)	2	37	1	24	16
Non-steroid pulse group (cases)	4	35	1	27	13

Table 2: Comparison of general clinical data

	Methylprednisolone pulse group	Non-steroid pulse group
Sex	28/12	26/14
Age (M)	25.40±13.24	26.95±12.44
Weight (Kg)	12.99±3.02	13.07±2.36
Temperature (°C)	39.05±0.80	39.12±0.71
Duration of fever (d)	4.78±1.21	5.08±1.02
Duration of nervous system involvement (d)	4.58±2.40	4.65±2.51
Hospitalization (d)	7.65±2.01	7.48±1.60

Table 3: Comparison of vital signs and laboratory findings on admission

	Methylprednisolone pulse group	Non-steroid pulse group
Heart rate (HR/min)	142.18±17.67	141.40±11.36
Respiratory rate (RR/min)	36.60±7.66	37.33±8.24
SBP (mmHg)	100.28±11.65	98.50±9.51
DBP (mmHg)	63.28±8.25	60.98±6.89
WBC (×10 ⁹ /L)	13.60±2.55	13.45±4.11
Hb (g/L)	120.88±11.96	117.60±12.98
PLT (×10 ⁹ /L)	222.30±82.61	237.43±87.08
CRP (mg/L)	8.46±6.11	9.94±8.53
GLU (mmol/L)	8.28±2.59	8.32±2.33

Note: SBP, Systolic blood pressure; DBP, Diastolic blood pressure

Table 4: Comparison of vital signs and laboratory findings 48-72h after hospitalization

	Methylprednisolone pulse group	Non-steroid pulse group
Heart rate (/min)	144.13±15.24	137.43±10.75 ^Δ
Respiratory rate (/min)	38.18±8.05	35.20±6.04 ^Δ
SBP (mmHg)	99.20±9.62	97.43±8.13
DBP (mmHg)	62.65±7.85	61.20±5.81
WBC (×10 ⁹ /L)	14.87±3.72	12.75±3.32 ^Δ
Hb (g/L)	120.70±10.88	118.13±12.30
PLT (×10 ⁹ /L)	230.98±95.02	234.80±76.71
CRP (mg/L)	9.25±9.50	8.97±5.77
GLU (mmol/L)	9.09±3.87	7.74±2.28 ^Δ

Note: SBP, Systolic blood pressure; DBP, Diastolic blood pressure; ^ΔP<0.05

Table 5: Treatment Results

	Cured	Improved	Death
Methylprednisolone pulse group	26 (100%)	0	0

DISCUSSION

Human is currently the only known natural host for EV71. Both patients and asymptomatic carriers are the important source of infection. Children younger than 10 years of age and especially younger than 5 years old are susceptible to

EV71 infection (Ma *et al.*, 2010). The pathogenesis of EV71 infection in the nervous system may be associated with virus-induced direct damage and immunologic injury (Huang *et al.*, 2006). Brainstem encephalitis was the major manifestation of EV71 nervous system involvement. In some children with brainstem

encephalitis, the disease may progress rapidly in a short time and serious complications will develop, such as pulmonary edema, pulmonary hemorrhage and circulatory failure. The mortality and disability rate is very high (Kim 2010, Solomon *et al.*, 2010). However, the mechanism underlying the virus infection-induced acute pulmonary edema and circulatory failure is not yet fully understood, although most scholars believe that it is neurogenic. Animal experiments and clinical cases of brain injury have confirmed that severe HFMD cases were often presented with NPE, and were associated with over-activation of the sympathetic (Groothuis *et al.*, 2010, (Sedý 2008) . It is possible that intestinal viruses invade brainstem to cause brainstem encephalitis and damage the dorsal nucleus of the vagus nerve and nucleus of the solitary tract, resulting in over activation of the sympathetic. As a result, a large amount of catecholamine is released within a short period of time. Vasoconstriction occurs and afterload resistance is increased. These in turn cause the distribution of large amount of blood to the heart and induce NPE (as suggested by "collision theory" and "percolation theory"), eventually leading to heart and lung failure, the main cause for death (Prager *et al.*, 2003). Taiwan scholars (Chang *et al.*, 2007) agreed with this theory and proposed staged management for EV71 infection. Their studies indicated that staged management is correlated with reduced mortality rate and does not affect the prognosis of children with central nervous system involvement. Therefore, it is crucial to fully understand disease characteristics, pay close attention to disease changes, detect symptom early and implement staged management to possibly reduce mortality.

EV71-caused severe HFMD cases mainly refer to those with nervous system involvement and cardiopulmonary failure, equivalent to Stage II - IIIA. The mortality rate for Stage IIIB patients is very high. Therefore, the key to cure severe EV71 infection is to prevent the progression of disease from Stage II and IIIA to IIIB. Current there is no effective treatment and the main approach is comprehensive symptomatic therapy. Some believed that large dose steroids might inhibit excessive inflammatory responses in children with severe HFMD, thus preventing disease progression. However Chen *et al.* (2007) have suggested that steroids cannot reduce mortality during staged therapy and didn't recommend their use. Moreover, there is no definite evidence to show that acute cardiorespiratory failure is due to direct infection, either infectious pneumonia and myocarditis or severe viral infection-caused systemic inflammatory responses (Wang *et al.*, 2009). It's unclear whether large dose steroid pulse therapy is effective for children at Stage II or III. In this study, all 80 patients with HFMD progressed to Stage II or IIIA. Among the 40 patients who received high-dose steroid pulse therapy, 2 died. The difference in the duration of fever, duration of nervous system involvement, duration of hospital stay, blood pressure,

and cure rate between the steroid group and non-steroid group was not statistically different ($p > 0.05$), suggesting that high-dose steroid pulse treatment for severe EV71 infection doesn't shorten the duration or improve the prognosis. Previous studies using EV71-infected animal models have also shown that steroid treatment had side effects and did not reduce mortality in severe cases. Steroids should be used with caution (Lin *et al.*, 2009), consistent with our findings.

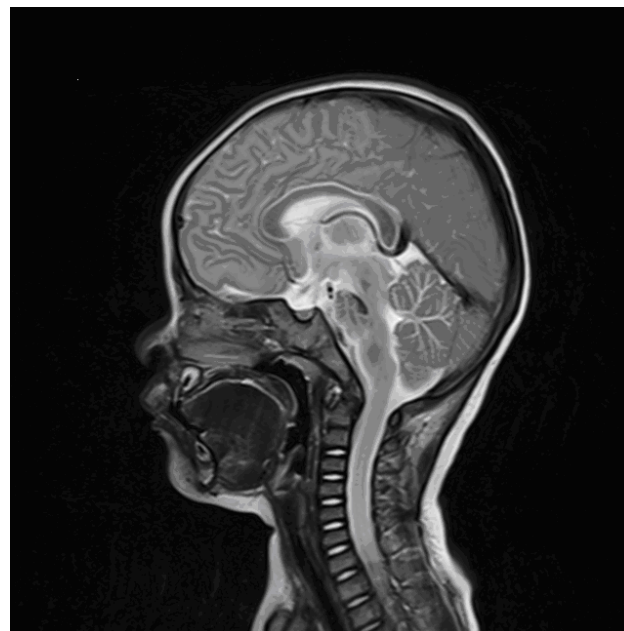


Fig. 4: An MRI study of patient 4 showed the diffuse signal shade long T2 in the brainstem.

It should be noted that high-dose steroid pulse therapy has side effects. Glucocorticoids can enhance the excitability of the central nervous system, increase the number of red blood cells and platelet counts. They can also increase the amount of neutrophils entering into the blood circulation, raise blood glucose and inhibit vasoconstriction induced by neurotransmitters such as epinephrine, norepinephrine, and angiotensin. Glucocorticoids also affect the metabolism of proteins, calcium, and phosphorus and cause complications such as Cushing's syndrome, osteoporosis, and aseptic necrosis of the femoral head. In this study, the heart rate, respiratory rate and blood glucose in children from the steroid pulse therapy group were significantly higher than those from the non-steroid treatment group ($p < 0.05$), probably due to the impact caused by large dose steroid. The mechanism underlying EV71 infection-caused NPE and heart damage is activation of the sympathetic (Groothuis *et al* 2010). One obvious sign indicating progression to heart and lung failure is dysfunction of the autonomic nervous system and abnormally high blood pressure and blood glucose. Previous studies have shown that hyperglycemia, increase in white blood cells and body weakness are the risk factors for pulmonary edema, of which hyperglycemia is

the most predictive factor (Chang *et al* (1999). Large dose steroid therapy is likely to enhance the excitability of the central nervous system and further increase heart rate, respiratory rate, blood glucose and white blood cells, hence aggravating the disease or affecting the diagnosis.

Other studies on EV71-infected patients and animal models (Chang *et al.*, 2006, Liu *et al.*, Liang *et al.*, 2014, Liang and Cao 2015, Liang and Guo 2015) have indicated that glucocorticoids can damage body's innate immune system and reduce the body's defense function, leading to more severe EV71 infection. Because severe EV71 infection is caused by viral damage of the nerve cells, use of glucocorticoids in the absence of antiviral drugs will potentially allow virus proliferation, thus aggravating the disease. In conclusion, high dose steroid therapy is more harmful than useful for the treatment of EV71 encephalitis.

In conclusion, our studies have suggested that high-dose steroid pulse therapy for EV71 encephalitis cannot shorten the course or improve the prognosis. In contrast, it has great side effects and may aggravate the condition or affect the diagnosis of the disease. Our results have also indicated that high-dose steroid therapy for EV71 encephalitis is not beneficial and thus not recommended. One limitation of this study is small sample size. Therefore in future studies we will collect and analyze more samples to further confirm our conclusions.

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