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ORIGINAL ARTICLE

The clinical value of aquaporin-4 in children with hand, foot, and mouth disease and the effect of magnesium sulfate on its expression: a prospective randomized clinical trial

Lei Zhu[1] · Hong Yin[2] · Haomiao Sun[3] · Tong Qian[4] · Junling Zhu[1] · Gongjian Qi[1] · Yin Wang[3] · Boxiang Qi[1]

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Abstract

To evaluate the clinical value of aquaporin-4 (AQP-4) in hand, foot, and mouth disease (HFMD) and to evaluate therapeutic efficacy of magnesium sulfate (MgSO4) and its effect on AQP-4 expression. Children with HFMD were divided into a common group, a severe group and a critical group according to Chinese guidelines; children in the critical group were further divided into two subgroups: routine treatment group and MgSO4 group. Outcome measures included systolic blood pressure (SBP), Heart rate (HR), the levels of AQP-4, interleukin-6 (IL-6), norepinephrine (NE), and neuron-specific enolase (NSE). Serum AQP-4, IL-6, NE, and NSE levels varied significantly among the critical, severe, and common groups before and after treatment. There were no significant differences in AQP-4 levels in cerebrospinal fluid (CSF) between the critical and severe groups before and after treatment; however, CSF AQP-4 levels in these two groups were higher than those in the common group before treatment. Serum and CSF AQP-4 levels in convalescence decreased significantly in the critical and severe groups. SBP, HR and serum AQP-4, IL-6, NE, NSE levels, but not CSF AQP-4 levels, were significantly lower in MgSO4 group than in the routine treatment group. AQP-4 in serum, but not in CSF, is a candidate biomarker for evaluating the severity and prognosis of HFMD; MgSO4 can provide protection on children with critical HFMD.

Keywords Hand, foot, and mouth disease · Aquaporin-4 · Magnesium sulfate · Cerebral injury

Introduction

Hand, foot, and mouth disease (HFMD) is an infectious disease caused by enteroviruses [1] that can cause cerebral edema and injury, further inducing catecholamine release, systemic inflammatory response, eventually leading to neurogenic pulmonary edema and other serious complications [2, 3]. Aquaporin-4 (AQP-4) has been shown to be closely related to cerebral edema [4]; nevertheless, its relationship with HFMD has not been reported. Magnesium sulfate (MgSO4) is used for treatment of asthma and bronchiolitis in pediatric patients [5]. MgSO4 can modify autonomic nervous system dysregulation [6] and provide neuroprotection by ameliorating neuroinflammation [7]. Nevertheless, it remains unclear as to whether MgSO4 ameliorates cerebral injury in children with HFMD. Therefore, we measured AQP-4 levels in children with various severities of HFMD and began an investigation into the mechanism of MgSO4 in treatment of critical HFMD.

✉ Boxiang Qi
xuzhoupicu@126.com

[1] Department of Intensive Care Unit, Xuzhou Children's Hospital of Xuzhou Medical University, Xuzhou, China

[2] Department of Pharmacy, The Affiliated Hospital of Xuzhou Medical University, Xuzhou, China

[3] Department of Infectious Disease, Xuzhou Children's Hospital of Xuzhou Medical University, Xuzhou, China

[4] Department of Clinical Laboratory, Xuzhou Children's Hospital of Xuzhou Medical University, Xuzhou, China

Materials and methods

Ethics statement

The study was conducted in accordance with the ethical principles stated in the Declaration of Helsinki and Chinese regulatory requirements, and the study was approved by the Hospital Medical Ethics Committee with the informed consent of the parents of the children.

Trial design and participants

This study was carried out in the Xuzhou Children's Hospital of Xuzhou Medical University. Children with HFMD admitted to the hospital beginning March 2017 were recruited. The inclusion and grouping criteria were formulated according to Chinese guidelines for the diagnosis and treatment of HFMD (2010 edition) [8] and expert consensus on clinical treatment of severe cases of enterovirus 71 (EV71) infection (2011 edition) [9]. The inclusion criteria included children with HFMD who were between the ages of 6 months and 6 years, and the disease course was between 2 and 5 days. The exclusion criteria were as follows: renal insufficiency, atrioventricular block, hypotension; hypocalcemia, congenital heart disease, chronic hepatitis, epilepsy, nephritis; thyroid dysfunctions, serious metabolic or hematological diseases, concurrent pneumonia, and concurrent enteritis. The selected children with HFMD were divided into a critical group (clinical stage 3), a severe group (clinical stage 2) and a common group (clinical stage 1, encephalitis excluded by CSF and other examinations), and the children in the critical group were further divided into the routine treatment group and MgSO4 group randomly, with a 1:1 ratio. Randomization was conducted using a random digital table. The investigators did not obtain prior notice of which participants were assigned to take either treatment.

Sample-size estimation

According to preliminary experiments and clinical experience, 25 participants per group and 26 participants per subgroup of critical HFMD were needed to have 90% power to identify significant differences and have a 2-sided alpha risk of 0.05. When the censored cases were considered, 120 patients (30 children in the common group, 30 children in the severe group, and 60 children in the critical group with 30 children in each subgroup) would be necessary.

Procedure

Three milliliters of venous blood were taken from the elbow before treatment in patients from the three groups, and the levels of AQP-4, IL-6, NE, and NSE were measured using commercial enzyme-linked immunosorbent assays (ELISA). These indexes were re-examined 7 days after beginning the treatment. Lumbar puncture was performed within 6 h of admission, and 2 ml CSF were collected to measure AQP-4 levels. Children with critical HFMD received treatment in accordance with the guidelines and expert consensus. In addition to the routine treatment group, the MgSO4 group received 50 mg/kg of intravenous bolus dose of MgSO4 once daily for 3–5 days [10]. Withdraw criteria for MgSO4 were as follows: substantial relief of the children's symptoms, SBP or HR below normal range, severe allergic reaction, and serum magnesium levels above 6 mg/dL [11]. The CSF AQP-4 levels were re-examined 10 days after beginning the treatment. HR and non-invasive SBP were recorded every hour, and the average of 1 day was used for analysis.

Statistical analysis

SPSS 20.0 software (IBM Corporation, USA) was used for data analysis. The measurement data were expressed as means ± standard deviations, differences among groups were analyzed by variance analysis, the Student *t* test was used for comparison between two groups, and demographic data was analyzed using the chi-square test. For all of tests, $P < 0.05$ was considered statistically significant.

Results

Enrollment and baseline characteristics

From March 2017 to October 2018, 273 children with HFMD were admitted to our hospital. Of these 273 children, 107 did not meet inclusion criteria; the parents of 49 children refused to participation. In total, 120 children were enrolled. Two children in the critical group died on the third day of admission. Three children were discharged without re-examination of serum indicators in the common group. Two children in the severe group and one child in the critical group refused to have their CSF re-examined, (Fig. 1). There were no significant differences in age, gender, or disease course among the three groups, as well as between the two subgroups. There were no significant difference in white blood cell (WBC) counts in CSF between the critical and severe groups ($P > 0.05$), but the WBC counts in both groups were higher than those in the common group ($P < 0.01$). CSF glucose levels in the critical group were higher than that in the common group ($P < 0.01$, Tables 1, 2). No severe adverse events related to drugs were observed.

Serum AQP-4, IL-6, NE, NSE, and CSF AQP-4 levels in the three groups

Before treatment, serum AQP-4, IL-6, NE, NSE, and CSF AQP-4 levels in the critical, and severe groups were significantly higher than those in the common group ($P < 0.05$), and all indexes (excepted for CSF AQP-4 levels) in the critical

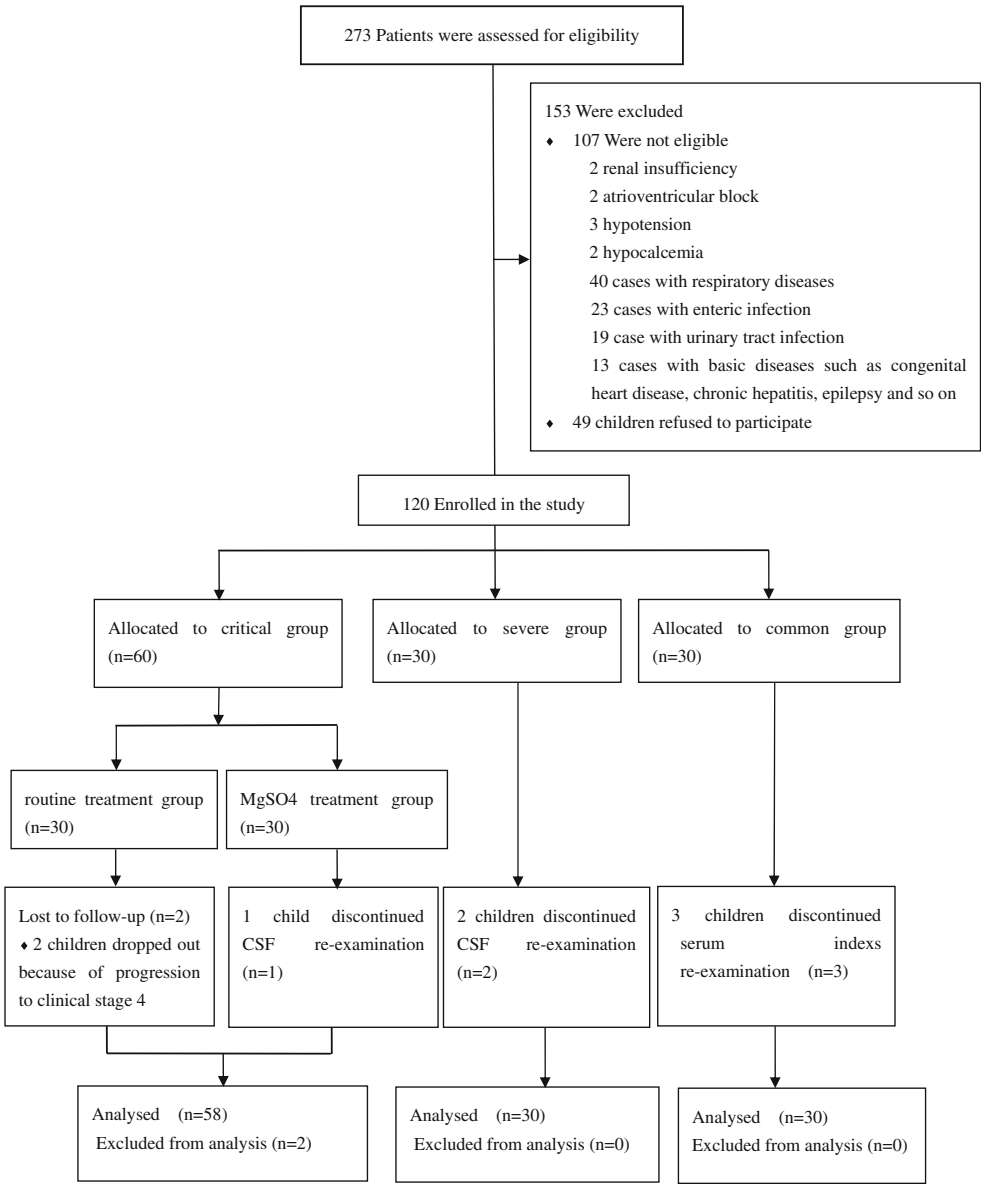

Fig. 1 Flow chart showing study enrolment

group were higher than those in the severe group ($P < 0.01$). After treatment, all these indexes were significantly lower in the severe and critical groups ($P < 0.01$). Compared with before treatment, serum NE and IL-6 levels were lower ($P < 0.05$), while serum AQP-4 and NSE levels showed no significant changes in the common group (Fig. 2).

Table 1 Baseline characteristics among three groups

Clinical data	Common group	Severe group	Critical group	F/χ^2	P
Age*, years	2.20 ± 0.89	2.04 ± 0.79	2.08 ± 0.80	0.310	0.734
Disease course*, days	3.45 ± 0.80	3.40 ± 0.79	3.16 ± 0.69	1.963	0.145
Sex (male/female)[&]	16/14	17/13	34/24	0.225	0.893
CSF WBC*, 10^6/L	6.03 ± 2.22	183.00 ± 86.74[a]	197.24 ± 82.30	86.205	0.000
CSF glucose*, mmol/L	3.62 ± 0.79	3.97 ± 0.73	4.42 ± 0.77 [a]	11.439	0.000
CSF protein*, g/L	0.31 ± 0.12	0.30 ± 0.09	0.31 ± 0.11	0.154	0.857
CSF chloride*, mmol/L	121.77 ± 5.04	124.30 ± 3.84	123.29 ± 4.28	2.557	0.082

CSF cerebrospinal fluid,

*Mean ± SD; [&] *n*. [a] $P < 0.01$, compared with common group

Table 2 General data between the two subgroups of the critical cases

Clinical data	Routine treatment group	MgSO4 group	t/χ^2	P
Age*,years	2.02 ± 0.79	2.13 ± 0.81	− 0.545	0.588
Disease course*, days	3.18 ± 0.66	3.13 ± 0.73	0.248	0.805
Sex (male/female)[&]	19/11	16/12	0.232	0.630
CSF WBC*, 10^6/L	194.14 ± 96.18	200.13 ± 68.43	− 0.272	0.787
CSF glucose*,mmol/L	4.45 ± 0.75	4.40 ± 0.80	0.209	0.835
CSF protein*,g/L	0.30 ± 0.13	0.32 ± 0.09	− 0.595	0.554
CSF chloride*, mmol/L	124.11 ± 4.35	122.53 ± 4.14	1.412	0.164

CSF cerebrospinal fluid
*Mean ± SD; [&] *n*

SBP and HR in the two subgroups of critical HFMD

There were no significant differences in HR and SBP between the two groups before treatment ($P > 0.05$), and HR and SBP significantly decreased after 1 day, 3 days, and 5 days of treatment ($P < 0.01$). Compared with the routine treatment group, the two indicators in the MgSO4 group were significantly alleviated after treatment ($P < 0.05$, Fig. 3).

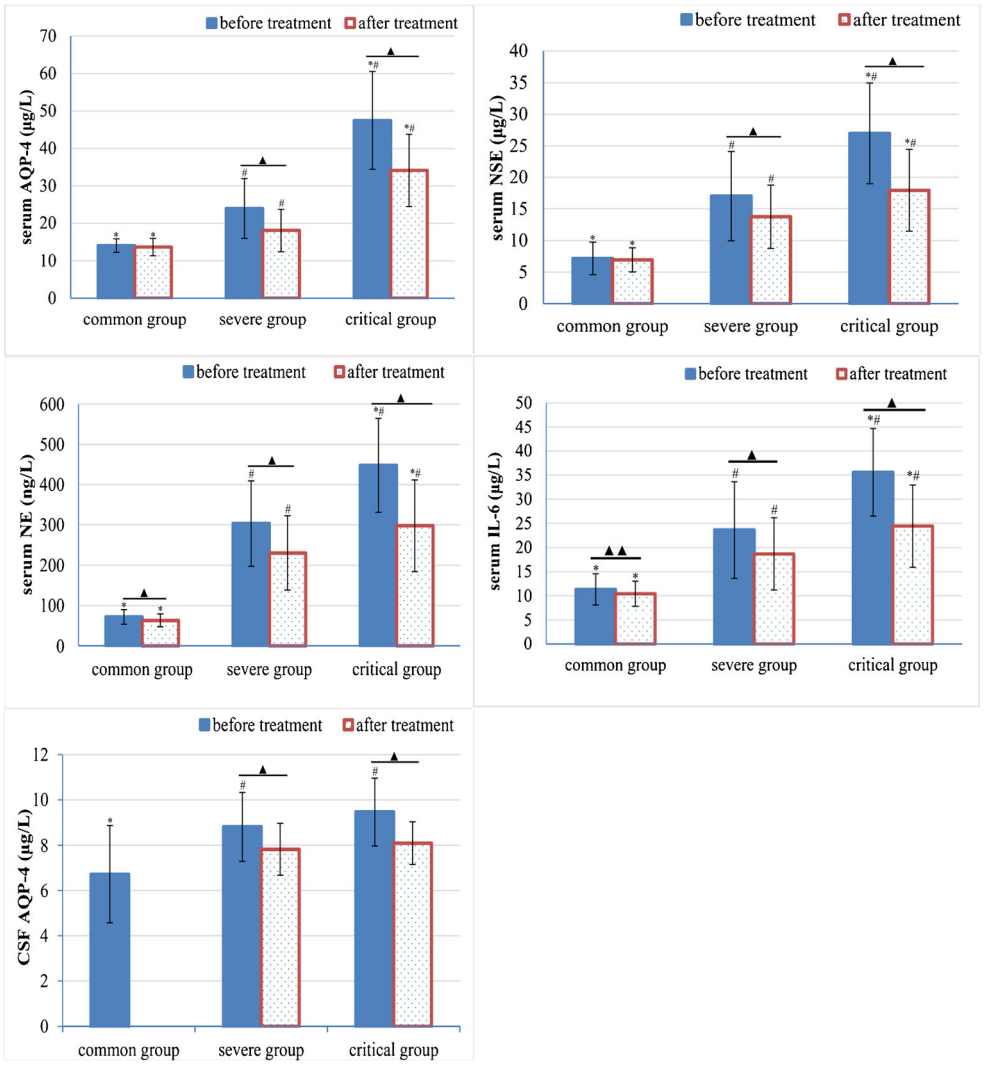

Fig. 2 Statistical analyses of serum AQP-4, IL-6, NE, NSE levels, and CSF AQP-4 expression among three groups before and after treatment. Compared with commom group, #$P < 0.01$; Compared with severe group, *$P < 0.01$. Compared with before treatment, ▲$P < 0.01$, ▲▲$P < 0.05$

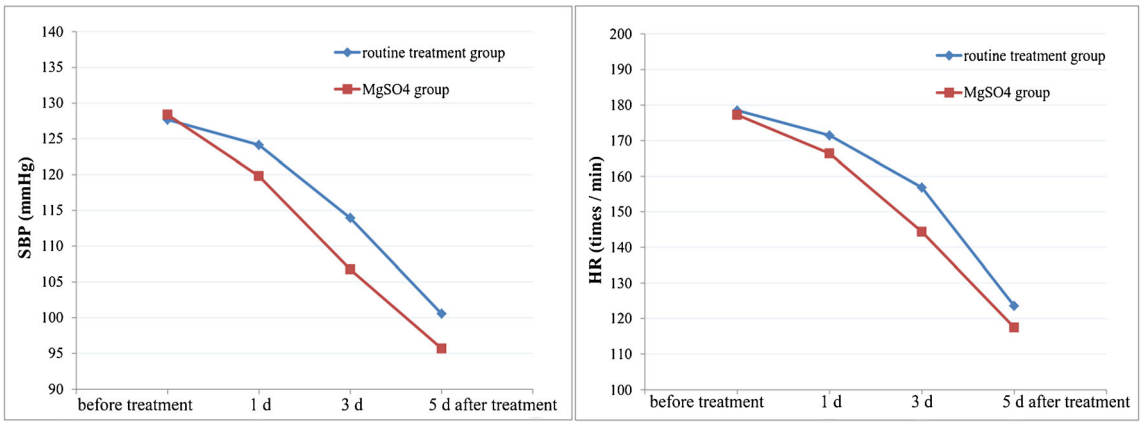

Fig. 3 Statistical analyses of SBP and HR in the two subgroups of critical HFMD patients before and after treatment

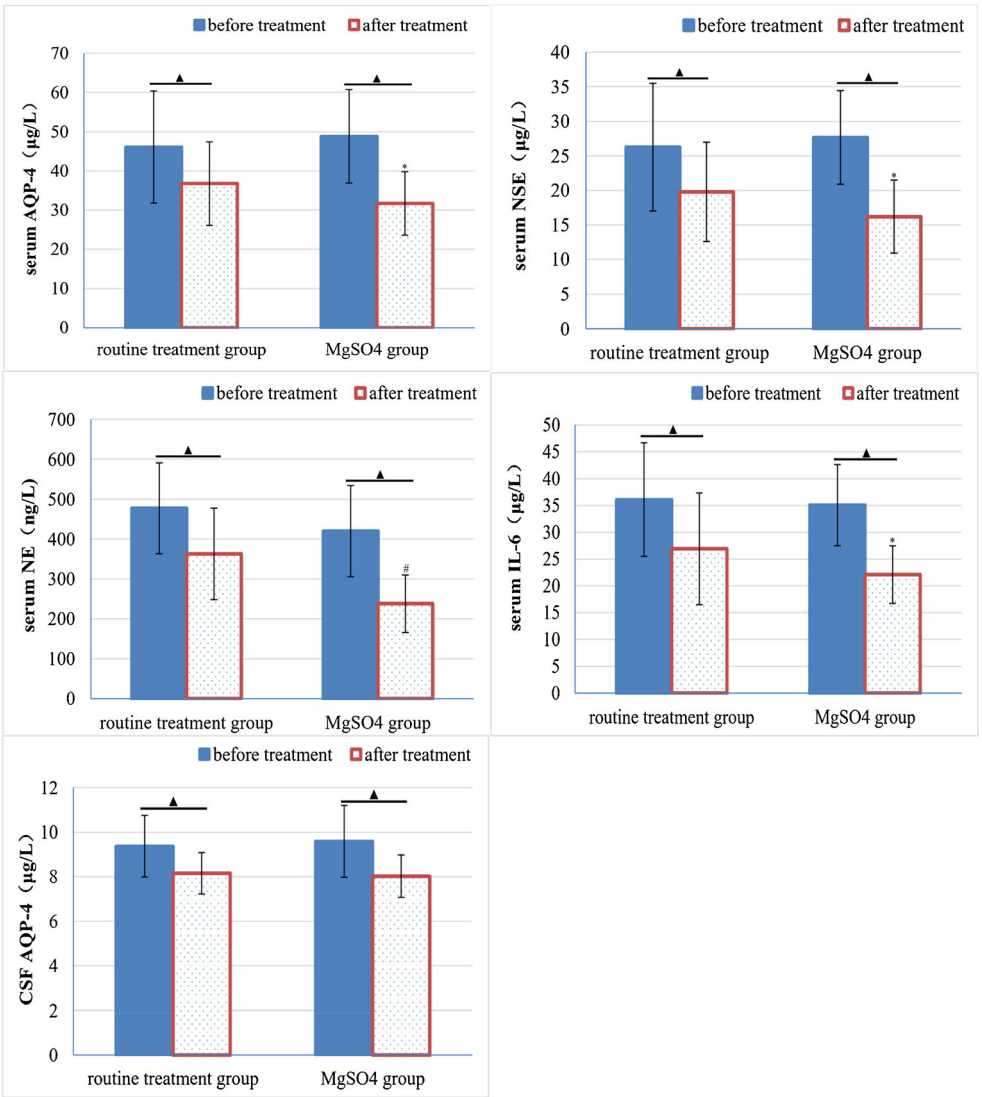

Fig. 4 Statistical analyses of serum AQP-4, IL-6, NE, NSE, and CSF AQP-4 in the two subgroups of critical HFMD before and after treatment. Compared with routine treatment group, $*P < 0.05$, $\#P < 0.01$; Compared with before treatment, $\blacktriangle P < 0.01$

Serum AQP-4, IL-6, NE, NSE, and CSF AQP-4 levels in the two subgroups of critical HFMD

Before treatment, there were no significant differences in serum AQP-4, IL-6, NE, NSE, and CSF AQP-4 levels between the two subgroups ($P > 0.05$), and all indexes significantly decreased in the two groups after treatment ($P < 0.01$). Compared with the routine treatment group, all indexes, excepted for CSF AQP-4 levels, in the MgSO4 group decreased significantly after treatment ($P < 0.05$, Fig. 4).

Discussion

Early identification of critical HFMD (clinical stage 3) and proper treatment remain the keys to reducing mortality [10]. NSE is a sensitive marker of cerebral injury [12], NE can be used as an indicator of catecholamine levels [13], and IL-6 plays an important role in the development of systemic inflammatory response syndrome [14].These three factors have been considered effective indicators for evaluating the severity of HFMD [15–17]. We found that serum NSE, NE, and IL-6 levels in children in the critical group were significantly higher than those in the other two groups, and these indicators were significantly lower after treatment; these results are consistent with previous studies [15–17]. Serum NE and IL-6 levels in convalescence decreased significantly in the common group, and this result may be related to the stress response caused by fear on admission. Aquaporins are a newly discovered family of membrane channel proteins. AQP-4 is closely related to cerebral edema and injury [18]. However, there are few studies on AQP-4 in HFMD patients. This study suggested that AQP-4 was involved in the pathological process of brain dysfunction in children with severe and critical HFMD; serum AQP-4 levels, but not CSF AQP-4 levels, could be used to estimate disease severity and prognosis. We found that serum AQP-4 levels were higher than those in CSF, possibly related to the expression of AQP-4 in the lungs, kidney, and other tissues [19, 20]. Pro-inflammatory cytokines could upregulate the expression of AQP-4 [21], and systemic inflammatory responses were observed in children with severe and critical HFMD [22]. Therefore, expression of AQP-4 in the lungs and other tissues would increase, which would affect the serum AQP-4 levels rather than CSF AQP-4 levels.

MgSO4 is commonly used in pediatrics, but studies of MgSO4 for treatment of critical HFMD have been rarely reported [6]. This study showed that MgSO4 significantly alleviated the HR and SBP and reduced serum NE, IL-6, and NSE levels, suggesting that MgSO4 effectively inhibits catecholamine storm and inflammation responses, providing neuroprotection. Some studies suggested that the neuroprotection by MgSO4 is mediated by lowering AQP-4 levels [23], but other studies showed different results [24]. This study showed that MgSO4 effectively reduced AQP-4 levels in serum but not in CSF in children with critical HFMD. The exact mechanism remains unclear, which may be related to the inhibition by MgSO4 on inflammatory responses. Nevertheless, this study suggests that the neuroprotection of MgSO4 may be not mediated via AQP-4 directly.

In conclusion, this study shows that the level of AQP-4 in serum, but not in CSF, is a candidate biomarker for evaluating the severity and prognosis of HFMD. MgSO4 can provide protection on children with critical HFMD; however, the neuroprotection of MgSO4 may be not mediated via AQP-4 directly.

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Author contributions Lei Zhu, Hong Yin, and Bixiang Qi conceived the study. Lei Zhu, Hong Yin, Haomiao Sun, and Boxiang Qi participated in the study design. Gongjian Qi and Junling Zhu participated in the statistical design. Tong Qian and Ying Wang conducted the study, including acquisition, detection, analysis, and interpretation of data. Gongjian Qi and Hong Yin completed the statistical analysis and data interpretation. All authors critically reviewed, edited, and approved the manuscript and made the decision to submit for publication. All authors assume responsibility for the accuracy and completeness of the data.

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Data availability All the data could be accessed by contacting the corresponding author at xuzhoupicu@126.com.

Compliance with ethical standards

The study was conducted in accordance with the ethical principles stated in the Declaration of Helsinki and Chinese regulatory requirements, and the study was approved by the Hospital Medical Ethics Committee with the informed consent of the parents of the children.

Competing interests The authors declare that they have no competing interests.

Ethical approval All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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