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Original Article

Significance of NSE and VCAM-1 in children with hand foot mouth disease combined with encephalitis

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Abstract: Hand-foot-mouth disease (HFMD) is caused by enterovirus with high morbidity and mortality once complicated with neural symptoms. Early diagnostic marker for evaluating severity is of critical importance. This study investigated the differential expression of serum or cerebro-spinal fluid (CSF) neuron specific enolase (NSE) and vascular cell adhesion molecule (VCAM)-1, and to elaborate their implication for early diagnosis and prognostic evaluation. The 93 patients were further divided into three groups based on disease conditions, including group A (31 cases without encephalitis), group B (41 cases with encephalitis) and group C (21 severe patients with encephalitis). Meanwhile, the recruited 32 healthy children was assigned as group D. ELISA was used to detect serum and CSF levels of NSE and VCAM-1 levels during acute and recovery stage. ROC analysis was employed to evaluate their significance in diagnosing complications and predicting prognosis. Multi-variable Logistic regression analysis revealed risk factors for disease progression. Both serum and CSF levels of NSE and VCAM-1 were higher with disease severity, and were lower in recovery period compared to acute phase ($P<0.05$ in all). ROC analysis showed areas for serum/CSF NSE and VCAM-1 in diagnosing HFMD with encephalitis as 0.908/0.866 and 0.958/0.897, plus sensitivity and specificity as 83.87%/75.81%, 90.32%/80.65%, 82.80%/87.10% and 81.72%/83.87%, respectively. Risk factors for HFMD complicated with encephalitis included elevated sensitivity of diagnosis, higher heart rate, and potentiated serum/ACSF levels of NSE or CXAM-1. Serum or CSF levels of NSE and VCAM-1 can reflect the severity of HFMD complicated with encephalitis, and may help to predict severe complications and prognosis.

Keywords: Hand-foot-mouth disease, complication, encephalitis, neuron specific enolase, vascular cell adhesion molecule-1

Introduction

Hand-foot-mouth disease (HFMD) is one acute infectious disease caused by enterovirus, and is mainly manifested as blisters in hands, mouth, hips and feet, commonly in pre-school children [1, 2]. HFMD normally has favorable prognosis. The complication of neural symptom, however, causes high morbidity and mortality, especially in those with brain stem encephalitis [3, 4]. Coxsackie virus A16 and enterovirus (EV) 71 are major pathogens for neural complications in HFMD. EV71 causes most severe HFMD cases due to its eosinophilic properties [5, 6]. EV71 commonly affects brain stem and spinal cord, with the former one as the most susceptible region. Currently little has been known about effective method and sensitive index evaluating neural damages caused by severe EC71 infection. Due to the lack of effective treatment at early phase, EV71 infection causes relatively high morbidity and mortality [7, 8]. Therefore the establishment of early diagnostic marker for predicting severity of disease is of critical importance. In clinics, neural electrophysiological examination and scale are often used in conjunction with clinical symptoms, and quantitative indexes for pathological changes. Neuron specific enolase (NSE) is one specific index reflecting the severity of

brain damage. It has relatively lower level in body fluids under normal conditions. Serum and CSF levels of NSE are known to be correlated with apoptotic number of neural cells [9, 10]. Its level thus can reflect the degree of neural system injury. Vascular cell adhesion molecule (VCAM)-1 can also reflect the degree of brain damage in viral encephalitis patients to certain extents, as previous studies showed elevated expression of VCAM-1 in serum and CSF samples from HFMD patients complicated with encephalitis [11, 12]. This study thus analyzed the differential expression of NSE and VCAM-1 in serum and CSF from HFMD patients complicated with encephalitis, and discuss their implications in early diagnosis and prognostic prediction. Multi-variant Logistic regression analysis was used to reveal risk factors for disease progression to provide evidences for prognostic prediction.

Materials and methods

Clinical information

A total of 93 HFMD children who were admitted in Qilu hospital from April 2012 to June 2015 were recruited. The age of patients ranges from five months to four years (average age: 2.15± 0.61 years). Among the 93 patients, there were 54 males and 39 females. The patients were further divided into three groups based on disease conditions, including group A (31 cases without encephalitis), group B (41 cases with encephalitis) and group C (21 severe patients with encephalitis). All patients had lab results and brain imaging for encephalitis diagnosis, in coupled with clinical neural symptoms. HFMD diagnosis was made in accordance with the guidance for diagnosis and treatment of HFMD. This study has been pre-approved by the ethical committee of our hospital and has obtained written consent forms from all participants' parents. Another 32 healthy patients who received lumbar anesthesia in surgery were recruited as group D (control group). No significant difference of age existed among all four groups ($P > 0.05$).

Sample collection and assays

4 ml fasted venous blood samples were collected during 24-hr acute phase and recovery period after surgery. Serum was collected by centrifugation. CSF (4 ml) was also collected by lumbar puncture, and was centrifuged at 14000 g for 15 min to collect the supernatant. ELISA approach was used to test NSE and VCAM-1 levels in serum and CSF samples using test kits (ADL, US). End-point method was used to test protein concentration of CSF using test kit (ADL, US). Rate transmission turbidity was used to describe serum immunoglobulin IgM and IgG levels. A retrospective analysis was performed to investigate all clinical information and lab results of patients. Multi-variant Logistic regression analysis was used to elaborate risk factors affecting disease progression.

ROC evaluation for the significance of NSE and VCAM-1 in complication diagnosis and prognosis evaluation

ROC analysis was performed to investigate the potential of NSE and VCAM-1 in serum and CSF in the complication diagnosis of HFMD and prognosis evaluation. Youden's index J was calculated as sensitivity + specificity-1. The best critical point was set as those levels having maximal tangency of J values, i.e. those with serum NSE > 16.3 μg/L, CSF NSE > 20.1 μg/L, serum VCAM-1 > 429.3 μg/L, and CSF VCAM-1 > 24.3 μg/L were determined as positive cases. Both specificity and sensitivity of these two indexes for predicting complications were analyzed.

Statistical methods

SPSS 19.0 software was used for the data statistical analysis in this study. The comparison of ratios was performed by chi-square test and corrected chi-square test. Kolmogorov-Smirnov test was performed for normality test. Those data fitted with normal distribution were expressed as mean ± standard deviation (SD), and were compared by analysis of variance among group means followed by LSD post-hoc test. ROC analysis was used to analyze the significance of single or combined index(es) for diagnosing HFMD related complications. A Logistic regression model was generated for combining assay using z-test. Multi-variant logistic regression analysis was performed to examine the risk factors affecting the progression of disease. A statistical significance was defined when $P<0.05$.

Results

Clinical information and relative indexes of participants

No statistically significant difference regarding age, sex or body weight has been identified

Table 1. Clinical indexes of participants

Index	Group A (n=31)	Group B (n=41)	Group C (n=21)	χ^2/F	*P*
Age (year)	2.09±0.47	2.23±0.69	2.31±0.72	1.233	0.23
Gender (M/F)	19/12	25/16	14/7	0.214	0.90
Body weight (kg)	11.93±3.02	12.31±3.23	12.63±2.95	0.828	0.41
Heart rate (per min)	122.93±9.11	123.61±12.38*	142.81±12.31$^{*,\Delta}$	6.694	0.00
Respiration rate (per min)	30.35±4.52	30.21±5.03	34.66±5.22$^{*,\Delta}$	3.169	0.00
CSF WBC count (10^6/L)	11.52±2.47	95.38±24.57*	147.83±35.21$^{*,\Delta}$	17.682	0.00
Positive rate of EV71 IgM (%)	12 (38.71)	38 (92.68)*	19 (90.48)*	13.038	0.00
Limb shakiness (n, %)	3 (9.68)	18 (43.90)*	14 (67.67)*	19.191	0.00
CSF protein (mg/L)	168.22±44.62	361.17±68.14*	386.33±71.22*	12.472	0.00

Note: *P<0.05 compared to group A; $^{\Delta}$P<0.05 compared to group B. Group A is equal to the HFMD without complications. Group B is equal to the HFMD with encephalitis. Group C is equal to patients with encephalitis.

Table 2. Expression level of NSE and VCAM-1

Group		Serum (μg/L)		CSF (μg/L)	
		NSE	VCAM-1	NSE	VCAM-1
Control group		7.22±1.47	245.36±22.31	8.16±2.31	10.22±2.3
Acute phase	A	10.36±3.11$^{\#}$	372.34±36.42$^{\#}$	11.63±3.67$^{\#}$	16.65±4.21$^{\#}$
	B	16.61±4.18$^{\#,*}$	486.22±42.31$^{\#,*}$	21.67±4.14$^{\#,*}$	28.97±5.13$^{\#,*}$
	C	21.63±5.12$^{\#,*,\Delta}$	502.33±55.11$^{\#,*,\Delta}$	28.13±4.85$^{\#,*,\Delta}$	31.22±5.87$^{\#,*,\Delta}$
Recovery period	A	9.22±1.67$^{\blacktriangledown}$	302.44±27.65$^{\blacktriangledown}$	10.03±2.43$^{\blacktriangledown}$	11.67±3.11$^{\blacktriangledown}$
	B	13.78±3.24$^{\#,*,\blacktriangledown}$	388.66±37.56$^{\#,*,\blacktriangledown}$	15.87±4.13$^{\#,*,\blacktriangledown}$	17.66±3.18$^{\#,*,\blacktriangledown}$
	C	15.68±4.31$^{\#,*,\blacktriangledown}$	402.21±38.26$^{\#,*,\blacktriangledown}$	19.68±4.72$^{\#,*,\blacktriangledown}$	22.35±3.41$^{\#,*,\blacktriangledown}$

Note: $^{\#}$P<0.05 compared to control group; *P<0.05 compared to group A; $^{\Delta}$P<0.05 compared to group B; $^{\blacktriangledown}$P<0.05 compared to acute phase in the same group.

among all groups (P > 0.05). The heart rate, respiration frequency and WBC count in CSF are all highest in group C, followed by group B and group A (P<0.05). Protein level in CSF, limb shakiness and IgM positive rate of EV71 were higher in group B or C compared to group A (P<0.05, **Table 1**).

Serum and CSF levels of NSE and VCAM-1

In acute stage, both NSE and VCAM-1 levels in serum or CSF samples were highest in group C, followed by group B and group A, and control group (P<0.05). During recovery period, NSE and VCAM-1 levels were significantly lower than acute phase (P<0.05) but were still higher than controls (**Table 2**).

ROC analysis for evaluating the significance of NSE and VCAM-1 in diagnosing HFMD complications

Using control (patients without complicates) and those patients with complicates as the research objects. ROC analysis was employed by using the NSE and VACM-1 as the dependent variable to evaluate the significance of serum/CSF levels of NSE or VCAM-1 in diagnosing complications. ROC results showed that both values had certain values in diagnosing HFMD complicated with encephalitis, with highest efficiency of CSF NSE level, followed by serum NSE, CSF CCAM-1, and serum VCAM-1 levels. Using those levels in HFMD patients without complications, and ROC curves from both indexes, Youden's index J was calculated as sensitivity + specificity-1. The best critical point was set as those levels having maximal tangency of J values, i.e. those with serum NSE > 16.3 μg/L, CSF NSE > 20.1 μg/L, serum VCAM-1 > 429.3 μg/L, and CSF VCAM-1 > 24.3 μg/L were determined as positive cases. Both sensitivity and specificity of two indexes were evaluated as shown in **Figures 1**, **2** and **Tables 3**, **4**.

Risk factor analysis of HFMD children complicated with encephalitis

Results showed fasted heart rate, higher CSF protein level and serum NSE/VCAM elevation as risk factors for HFMD complicated with

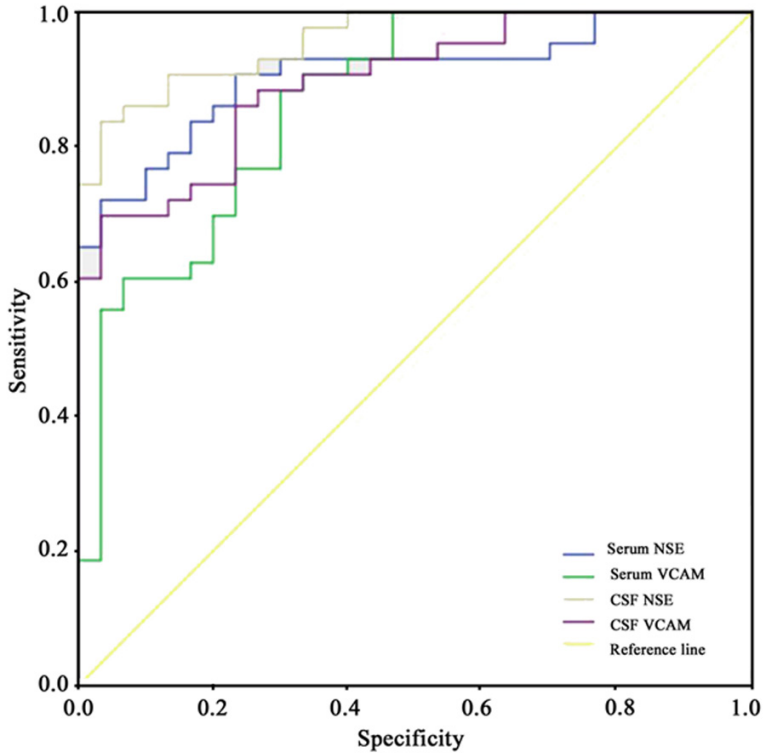

Figure 1. ROC analysis of NSE and VCAM-1 indexes in HFMD complicated with encephalitis.

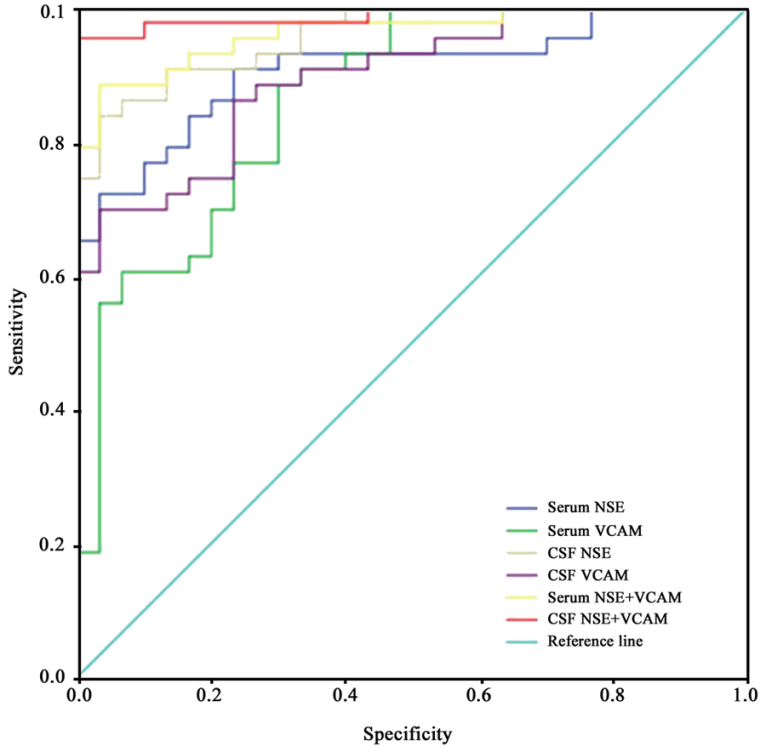

Figure 2. ROC analysis of NSE combined with VCAM-1 indexes in HFMD diagnosis.

encephalitis (P<0.05, **Table 5**). Meanwhile, using the progression into severe cases as the dependent variable, all clinical indexes were recruited as independent variables in multi-variant Logistic regression (**Tables 6**, **7**).

Discussion

Most HFMD cases complicated with encephalitis are induced by EV71 infection, whose pathogenesis mechanism, however, has not been fully illustrated. Previous studies indicated the possible correlation with retrograde axonal transport for CNS injury [7, 8]. Currently few methods can be used to evaluate CNS injury caused by severe viral infection in effective manners. The progression of HFMD can be divided into three phases, during which encephalitis or encephalomyelitis play a critical role during the progression into severe cases [11, 13]. Currently there were fewer objective indexes predicting the neural injury condition of HFMD. The searching for risk factors as early markers for critical cases and disease progression is thus of importance for treating severe patients [14, 15].

Currently used biological markers evaluating neuronal injury include NSE and S-100β proteins. NSE is one specific marker for neuronal damage, as CSF level of NSE directly reflect the degree of neuronal injury in high accuracy [16, 17]. NSE level at early phase is correlated with prognosis, as high NSE level usually indicates unfavorable prognosis. In HSV encephalitis patients, CSF NSE level during acute

Table 3. Area under the curve of ROC for NSE and VCAM-1

Test parameter	Area	Standard error[a]	Regression Sig.[b]	95% CI	
				Lower limit	Upper limit
Serum NSE	.908	.034	.000	.840	.975
Serum VCAM	.866	.043	.000	.782	.950
CSF NSE	.958	.020	.000	.920	.997
CSF VCAM	.897	.035	.000	.829	.965
Serum NSE+VCAM	.963	.020	.000	.924	1.000
CSF NSE+VCAM	.988	.011	.000	.966	1.000

Note: a, under non-parametric hypothesis; b, null hypothesis: area =0.5.

Table 4. Test indexes of NSE and VCAM-1

Index	Critical value μg/L	Sensitivity/%	Specificity/%	PPV	NPV
Serum NSE	> 16.3	83.87 (52/62)	82.80 (77/93)	79.03 (49/62)	70.5 (31/44)
Serum VCAM	> 429.3	75.81 (47/62)	87.10 (81/93)	82.3 (51/62)	75.6 (31/41)
CSF NSE	> 20.1	90.32 (56/62)	81.72 (76/93)	85.5 (53/62)	77.5 (31/40)
CSF VCAM	> 24.3	80.65 (50/62)	83.87 (78/93)	77.4 (48/62)	68.9 (31/45)
Serum NSE+VCAM	> 12.3, > 498.4	72.6 (45/62)	76.34 (71/93)	71.0 (44/62)	63.3 (31/49)
CSF NSE+VCAM	> 25.2, > 27.5-	75.8 (47/62)	75.27(70/93)	74.2 (46/62)	66.0 (31/47)

Table 5. Risk factor analysis for disease progression of HFMD complicated with encephalitis by using logistic regression analysis

Related factor	β	$S_{\bar{x}}$	Wald χ^2	*P*	OR	95% CI
Faster heart rate	0.696	0.278	6.247	0.00	2.006	1.162~3.462
CSF protein	1.347	0.415	10.512	0.00	3.846	1.704~8.682
Serum NSE	1.163	0.319	4.589	0.00	3.200	1.711~5.982
Serum VCAM-1	1.113	0.470	5.134	0.00	3.043	1.212~7.640

Table 6. Univariate regression analyses for prognosis of the HFMD children complicated with encephalitis

Variable		OR (95% CI)	*P* values
Serum NSE	> 16.3 ≤ 16.3	1.711-5.982 (3.2)	<0.001
Serum VCAM	> 429.3 ≤ 429.3	1.21-7.64 (3.043)	<0.001
CSF NSE	> 20.1 ≤ 20.1	1.532-6.094 (2.861)	<0.001
CSF VCAM	> 24.3 ≤ 24.3	1.804-8.943 (4.132)	<0.001

stage is related with pathological changes. Some studies showed the important role of VCAM-1 in pathology mechanism of brain injury, as it is related with visible brain lesion by cerebrovascular MRI imaging, plus elevated VCAM-1 level after brain ischemia-reperfusion, and alleviation of brain damage by VCAM-1 monoclonal antibody [18, 19]. During the occurrence and progression of viral encephalitis, VCAM-1 is probably involved. However, few studies have been performed regarding the role of VCAM-1 in the diagnosis and disease prediction of HFMD patients complicated with encephalitis [20-22]. This study showed higher serum/CSF levels of NSE and VCAM-1 in HFMD patients complicated with severe encephalitis, as compared to those HFMD patients without encephalitis, which had higher levels than healthy age-controlled children. During recovery period, NSE and VCAM-1 levels were significantly decreased, but were still higher than control group. All these data supported the role of serum or CSF levels of NSE and VCAM-1 in reflecting the injury degree of neural system and disease progression.

ROC analysis was further performed to evaluate the significance of serum/CSF levels of NSE and VCAM-1 in diagnosing complications and prognosis prediction. Results showed certain values of both indexes in diagnosing HFMD complicated with encephalitis, with better efficacy in CSF NSE, followed by serum NSE, CSF

Table 7. Multivariate backward stepwise regression analyses for prognosis of the HFMD children complicated with encephalitis

Variable		Unadjusted OR (95% CI)	*P* values
Serum NSE	> 16.3	1.424-5.314 (2.9)	<0.001
	≤ 16.3		
Serum VCAM	> 429.3	1.03-6.78 (2.804)	<0.001
	≤ 429.3		
CSF NSE	> 20.1	1.318-5.674 (2.732)	<0.001
	≤ 20.1		
CSF VCAM	> 24.3	1.572-8.207 (3.694)	<0.001
	≤ 24.3		

VCAM and serum VCAM. Serum NSE and VCAM-1 had relatively higher sensitivity and specificity when diagnosing HFMD complicated with encephalitis, suggesting certain values of NSE and VCAM-1 in assisting the diagnosis of HFMD complicated with encephalitis, and in predicting severe complication and patient prognosis. The combined assay of two indexes had better diagnostic efficacy, indicating that combined assay could reflect the disease condition in early phase of HFMD complicated with encephalitis, and improve both sensitivity and accuracy in disease diagnosing, thus benefiting the early diagnosis of HFMD complicated with encephalitis. The monitor of serum and CSF biomarkers reflecting HFMD complicated with encephalitis help to monitor the condition of early phase complication in neural systems. Multi-variant Logistic regression analysis in our study revealed various risk factors for HFMD complicated with encephalitis, including faster heart rate, higher CSF protein and elevated serum levels of NSE and VCAM, suggesting the requirement of close monitor for patient's heart rate and clinical manifestation, which can benefit early diagnosis and timely treatment. For those patients with elevated CSF protein and serum NSE/VCAM levels, the disease condition should be closely monitored to give timely treatment.

Both serum and CSF levels of NSE and VCAM-1 in HFMD complicated with encephalitis children can reflect the disease severity to certain extents, and may predict severe complication and unfavorable prognosis.

Disclosure of conflict of interest

None.

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