

Significance of detecting cerebrospinal fluid ev71 antibody on severe hfmd with encephalitis complication.

Weiwei Dai^{1#}, Jun Ma^{2#}, Weiwei Feng¹, Tingting Lin¹, Lijing Zhou¹, Wei Qin¹, Xuyang Wang^{3*}, Zhongqiu Lu^{1*}

¹Department of Critical Care Medicine, the First Affiliated Hospital of Wenzhou Medical University, Wenzhou, Zhejiang, PR China

²Department of Pediatrics, Yan'an Affiliated Hospital of Kunming Medical University, Kunming, Yunnan, PR China

³Department of Neurosurgery, Shanghai Jiaotong University Affiliated 6th People's Hospital, Shanghai, PR China

#These authors contributed equally to this paper

Abstract

Objective: This paper aimed to analyze the importance of cerebrospinal fluid (CSF) EV71 antibody on the diagnosis of patients with severe hand, foot, and mouth disease (HFMD) accompanied with encephalitis.

Method: Data were collected from 224 children patients with severe HFMD from March 2015 to March 2017. According to the occurrence and types of encephalitis, the participants were divided into control group (no encephalitis, n=72), bacterial encephalitis group (HFMD with bacterial encephalitis, n=86), and viral encephalitis group (HFMD with viral encephalitis, n=66). Serum and CSF EV71 antibody levels were detected. The sensitivity and specificity of CSF EV71 antibody for the early prediction of HFMD accompanied with encephalitis were measured using receiver operating characteristic (ROC) curves. The relationship of serum EV71 antibody on severe HFMD accompanied with encephalitis was determined based on logistic multivariate regression analysis.

Results: The serum and CSF EV71 antibody levels of the bacterial encephalitis and viral encephalitis groups were significantly higher than those of the control group ($P<0.05$). The bacterial encephalitis and viral encephalitis groups showed no statistical difference in their serum and CSF EV71 antibody levels ($P>0.05$). ROC curves showed the relatively high sensitivity and specificity of serum and CSF EV71 antibody for the early prediction of severe HFMD accompanied with encephalitis ($P<0.05$). Results of logistic multivariate regression analysis showed that when encephalitis complication was used as the dependent variable, all the impact factors of serum and CSF EV71 antibody exhibited statistical significance ($P<0.05$).

Conclusions: Severe HFMD with encephalitis complication can cause brain tissue damage among children and induce the sharp growth of serum and CSF EV71 antibody levels. The detection of serum EV71 antibody is characteristic of simple operation and small trauma. Thus, this antibody plays an important role in the early prediction of severe HFMD with encephalitis complication.

Keywords: CSF EV71 antibody, Severe HFMD, Encephalitis, Prediction.

Accepted on September 28, 2017

Introduction

Hand, foot, and mouth disease (HFMD) is an infectious disease caused by intestinal tract 71-type virus and coxsackievirus A16. Abundant vesicular exanthemas at hand, foot, mouth, and hip are the main clinical symptoms [1,2]. This disease easily attacks preschoolers and often has favorable prognosis. However, children with severe HFMD easily suffer from pneumonia, encephaledema, circulatory disturbance, and other complications [3]. Encephalitis is one of the most serious

complications of severe HFMD. Early recognition of encephalitis risk and scientific treatment is important to protect the quality of life of affected children. Nevertheless, the existing clinical diagnosis methods of severe HFMD with encephalitis complication mainly depend on lumbar cerebrospinal fluid (CSF) examination, a complicated and time-consuming examination that could not meet the requirements of early diagnosis [4]. In this paper, the importance of the key factor of cerebral injury, CSF EV71 antibody, on the early prediction of severe HFMD with

encephalitis complication was analyzed to provide references for clinical diagnosis.

Data and Methods

General data

Data were collected from 224 children patients with severe HFMD in our hospital from March 2015 to March 2017. According to the occurrence and types of encephalitis, the participants were divided into the control group (no encephalitis, n=72), bacterial encephalitis group (HFMD with bacterial encephalitis, n=86), and viral encephalitis group (HFMD with viral encephalitis, n=66). All these patients were all diagnosed according to the HFMD diagnosis guideline issued by the Ministry of Health; patients with severe HFMD but with other types of infection were eliminated. The three groups showed no statistically significant difference in terms of age, course of disease, body weight, and gender proportion ($P>0.05$). All three groups are comparable in terms of general data (Table 1). This paper was approved by the Medical Ethics Committee of our hospital, and the guardians of patients signed an informed consent.

Table 1. Comparison of general clinical data of three groups (n%).

Index	Control group (n=72)	Bacterial encephalitis group (n=86)	Viral encephalitis group (n=66)	P
Age (month)	28.39 ± 4.17	29.05 ± 4.24	28.58 ± 4.22	>0.05
Course of disease (day)	1.81 ± 0.26	1.89 ± 0.31	1.85 ± 0.25	>0.05
Body weight (kg)	12.64 ± 2.33	12.95 ± 2.24	12.79 ± 2.51	>0.05
Gender (male)	43 (59.72)	53 (61.63)	39 (59.09)	>0.05
Gender (female)	29 (40.28)	33 (38.37)	27 (40.91)	

Detection method

On the second day of sampling (before the therapy), 2 mL of elbow vein blood and 1.5 mL of CSF were collected from each patient on an empty stomach. Serum and CSF EV71 antibody levels were tested by double antibody enzyme-linked immunosorbent assays. The kits were bought from Shanghai Genetimes ExCell Biotech Co., Ltd. Test methods were adopted from related literature. The above indexes were tested by two laboratory physicians under double-blind conditions. Mean values were used as the final test results.

Research method

The differences in serum and CSF EV71 antibody were compared among the three groups. The sensitivity and specificity of serum and CSF EV71 antibody for the early prediction of severe HFMD with encephalitis complication were calculated by receiver operating characteristic (ROC) curves. In addition, the relationship between serum EV71

antibody and severe HFMD with encephalitis complication was determined by logistic multivariate regression analysis.

Statistical analysis

All clinical research data were analyzed by SPSS18.0. Enumeration data were expressed in (n/%) and examined by χ^2 -test. Measurement data were expressed in ($\bar{x} \pm s$). Independent sample t-test was used when the data meet the homogeneity of variance, whereas the calibration t-test is used when heterogeneity was achieved. The relationship of EV71 antibody and severe HFMD with encephalitis complication was determined by logistic multivariate regression analysis. $P<0.05$ indicates statistically significant difference.

Results

Comparison on serum and CSF EV71 antibody level

The serum and CSF EV71 antibody levels of bacterial encephalitis and viral encephalitis groups were significantly higher than those of the control group ($P<0.05$). However, no statistically significant difference was found between the bacterial encephalitis and viral encephalitis groups ($P>0.05$). The results are listed in Table 2.

Table 2. Comparison of serum and CSF EV71 antibody level of three groups ($x \pm s$).

EV71 antibody (ng/mL)	Control group (n=72)	Bacterial encephalitis group (n=86)	Viral encephalitis group (n=66)	P
Serum	11.59 ± 3.24	40.35 ± 1.96*	39.71 ± 2.08 [†]	<0.05
CSF	12.59 ± 3.13	42.76 ± 4.84*	43.08 ± 4.91 [†]	<0.05

Note: * $P<0.05$ is relative to the control group.

Analysis of prediction values

ROC curves revealed the relatively high sensitivity and specificity of serum and CSF EV71 antibody for the early prediction of severe HFMD with encephalitis complication ($P<0.05$). Data are shown in Table 3.

Table 3. Clinical value of serum and CSF EV71 antibody for early prediction of severe HFMD with encephalitis complication.

Index	Threshold	AUC	Sensitivity (%)	Specificity (%)
CSF EV71 antibody	28.75 ng/mL	0.795	87.83	90.52
Serum EV71 antibody	26.82 ng/mL	0.734	85.16	89.33

Analysis of influencing factors

Results of multivariate regression revealed that the influencing factors of serum and CSF EV71 antibody were statistically

significant when encephalitis complication was used as the dependent variable ($P < 0.05$) (Table 4).

Table 4. Logistic multivariate regression results.

Factor	β	SE (β)	Wald χ^2	P	OR	95% CI	
CSF EV71 antibody	1.823	0.295	38.188	0.000	6.190	3.472–11.036	
Serum antibody	EV71	1.799	0.217	68.729	0.000	6.044	3.950–9.247

Discussion

Most patients with HFMD have self-limited development. After onset, the disease progresses and is accompanied by fever. Most patients can recover within 1 week and experience conditions, such as temperature reduction and deflorescence [5]. However, some patients with severe HFMD experience rapid disease progression and suffer from cephalomeningitis, encephalitis, encephalomyelitis, and other serious complications within 1-4 days after the onset. The death rate is relatively high, and the surviving patients have multiple sequelae. The recent spread of HFMD epidemic in multiple provinces in China requires an intensified HFMD prevention [6].

Some common pathopoiesis of HFMD include intestinal tract 71-type virus and coxsackievirus A16. Both are characteristics of high neurotropic and are the main causes of severe complications of the nervous system. Researchers discovered that patients with severe HFMD have higher risks of tachycardia, emesis, muscle clonus, agitation, and drowsiness than patients with non-severe HFMD, thereby confirming the above conclusions [7]. Furthermore, patients with severe HFMD have high risk of encephalitis. Brain stem encephalitis is an important stage of severe HFMD progress and is the precursor manifestation of pulmonary edema, pneumorrhagia, and circulatory failure. Therefore, early diagnosis of severe HFMD with encephalitis complication is important to avoid mental deficiency and extremity disability of patients.

The pathophysiological changes of early encephalitis are mainly manifested by cerebral vessel peripheral nerve necrosis. With the development of the disease, extensive necrosis and cumulative local phenomenon have become the main causes of subsequent clinical manifestations, including meningeal irritation sign, headache, and fever [8]. Researchers discovered that many inflammatory cells can access brain tissues through blood vessel endothelial system and subsequently induce the generation and development of nervous system impairment. As an important factor of controlling inflammatory cells, EV71 antibody might play an important role in the generation of encephalitis. In this paper, the serum and CSF EV71 antibody levels of patients with severe HFMD with encephalitis complication were compared with those of patients with severe HFMD with different complications [9]. Results revealed the significantly increased serum and CSF EV71 antibody levels of the bacterial encephalitis and viral encephalitis groups. No

statistically significant difference was found between these two groups, indicating that impaired endothelial function is one of the important pathogeneses of encephalitis. EV71 antibody can resist secondary impairments caused by direct and indirect impaired endothelial functions through the following mechanisms. As the heparin-binding growth factor of the specificity of vascular endothelial cells, EV71 antibody can promote the division and proliferation of vascular endothelial cells, induce *in-vivo* angiogenesis, and enhance vasopermeability, thus improving microvessel and collateral circulation [10]. Therefore, the increased EV71 antibody level reflects impaired endothelial functions and seriously damaged blood-brain barrier. Researchers reported that EV71 antibody might be involved in the pathogenesis of HFMD with encephalitis complication. These findings can provide certain references for the early diagnosis of encephalitis.

The ROC curves and the results of logistic multivariate regression analysis revealed that serum and CSF EV71 antibody are important influencing factors of severe HFMD with encephalitis complication. The occurrence risk of encephalitis can be predicted according to the EV71 antibody level. Serum EV71 antibody and CSF EV71 antibody can both be used to predict severe HFMD with encephalitis complication. This method could be widely used in predicting severe HFMD with encephalitis complication because lumbar CSF collection is painful and has limited safety. In addition, researchers discovered that the changes in serum EV71 antibody are related to the changes in HFMD to some extent. This finding provides a new idea for HFMD prediction. The prognosis correlation between EV71 antibody and HFMD must be established by the use of large sample size and multi-center analysis.

Conclusion

Children with severe HFMD and encephalitis complications have increased serum and CSF EV71 antibody levels. The risks of encephalitis complication can be predicted effectively according to the level of serum EV71 antibody. This finding offers reliable references for early clinical diagnosis and requires further attention.

References

1. Ziemka-Nalecz M, Jaworska J, Zalewska T. Insights into the neuroinflammatory responses after neonatal hypoxia-ischemia. *J Neuropathol Exp Neurol* 2017; 76: 644-654.
2. Yu G, Hua ZH, Li Z, Wei LT. Hepatocyte growth factor shows benefits for patients with chronic renal failure induced type 2 diabetes. *Biomed Res-India* 2017; 28: 3837-3844.
3. Fraga VG, Carvalho MDG, Caramelli P, de Sousa LP, Gomes KB. Resolution of inflammation, n-3 fatty acid supplementation and Alzheimer disease: A narrative review. *J Neuroimmunol* 2017; 310: 111-119.
4. Rule R, Baschar H, Cordiviola C, Prozzi G, Farina OH. Pharmacokinetics and tissue penetration of ceftazidime

- administered during hysterectomy and oophorectomy in canine females. *Lat Am J Pharm* 2014; 33: 1579-1582.
5. Kim JE, Heo JH, Kim HO, Song SH, Park SS, Park TH, Ahn JY, Kim MK, Choi JP. Neurological Complications during Treatment of Middle East Respiratory Syndrome. *J Clin Neurol* 2017; 13: 227-233.
 6. Guo N, Lin G. Effects of nonsurgical periodontal therapy on serum inflammatory factor levels in patients with chronic kidney disease and periodontitis. *Biomed Res-India* 2017; 28: 3899-3902.
 7. Arai T, Terao-Muto Y, Uchida S, Lin C, Honda T, Takenaka A, Ikeda F, Sato H, Yoneda M, Kai C. The P gene of rodent brain-adapted measles virus plays a critical role in neurovirulence. *J Gen Virol* 2017; 98: 1620-1629.
 8. Liu L, Ke F, Li C, Zhang X, Pi C, Wang L, Ke S, Luo Y, Zhong Z. Multivesicular liposomes loading with ursolic acid enhanced the in vitro antitumor activity on hepatic carcinoma cells. *Lat Am J Pharm* 2017; 36: 758-766.
 9. Pereira LP, Villas-Bôas R, Scott SSO, Nóbrega PR, Sobreira-Neto MA, Castro JDV, Cavalcante B, Braga-Neto P. Encephalitis associated with the chikungunya epidemic outbreak in Brazil: report of 2 cases with neuroimaging findings. *Rev Soc Bras Med Trop* 2017; 50: 413-416.
 10. Singanallur NB, Pacheco JM, Arzt J, Stenfeldt C, Fosgate GT, Rodriguez L, Vosloo W. Efficacy of a high potency O1 Manisa monovalent vaccine against heterologous challenge with foot-and-mouth disease virus of O/SEA/Mya-98 lineage in sheep. *Antiviral Res* 2017; 145: 114-122.

***Correspondence to**

Zhongqiu Lu

Department of Critical Care Medicine

The First Affiliated Hospital of Wenzhou Medical University

Wenzhou

PR China