Journal of Hainan Medical University

http://www.hnykdxxb.com

Correlation of serum NSE and S100β levels with inflammatory response and immune response in children with hand−foot−mouth disease complicated by encephalitis

Qiao−Li Chai✉

Department of Pediatrics, Yulin Third Hospital in Shaanxi Province, Yulin City, Shaanxi Province, 719000

ARTICLE INFO

Article history:
Received 12 Jun 2017
Received in revised form 19 Jun 2017
Accepted 3 Jul 2017
Available online 14 Jul 2017

Keywords:
Hand-foot-mouth disease
NSE
S100β
Inflammatory response
Immune response

ABSTRACT

Objective: To study the correlation of serum NSE and S100β levels with inflammatory response and immune response in children with hand-foot-mouth disease (HFMD) complicated by encephalitis. **Methods:** Children who were diagnosed with hand-foot-mouth disease in Yulin Third Hospital between May 2015 and February 2017 were selected, children who were combined with central nervous system were selected as severe group, and children who were not combined with central nervous system were selected as mild group; children who received physical examination during the same period were selected as the control group. Serum was collected to determine the contents of NSE, S100β and inflammatory response mediators, and peripheral blood was collected to determine the contents of T cell subsets and NK cells. **Results:** Serum NSE and S100β levels of severe group and mild group were significantly higher than those of control group, and serum NSE and S100β levels of severe group were significantly higher than those of mild group; serum TNF-α, IL-6, IL-10, IL-17 and PCT levels as well as peripheral blood HLA-DR$^+$CD4$^+$, HLA-DR$^+$CD8$^+$, CD38$^+$CD4$^+$ and CD38$^+$CD8$^+$ levels of severe group and mild group were significantly higher than those of control group while peripheral blood CD3$^+$T cell, CD4$^+$T cell, CD8$^+$T cell and NK cell levels were significantly lower than those of control group; serum TNF-α, IL-6, IL-10, IL-17 and PCT levels as well as peripheral blood HLA-DR$^+$CD4$^+$, HLA-DR$^+$CD8$^+$, CD38$^+$CD4$^+$ and CD38$^+$CD8$^+$ levels of severe group were significantly higher than those of mild group and positively correlated with NSE and S100β levels while peripheral blood CD3$^+$T cell, CD4$^+$T cell, CD8$^+$T cell and NK cell levels were significantly lower than those of mild group and negatively correlated with NSE and S100β levels. **Conclusion:** The increase of serum NSE and S100β levels in children with HFMD complicated by encephalitis is closely related to inflammatory response activation and immune response disorder.

1. Introduction

Hand-foot-mouth disease (HFMD) is a common acute infectious disease in childhood that is caused by a variety of enterovirus infections, and the enterovirus 71 and coxsackie A16 are the most common[1,2]. Common symptoms of HFMD include fever and herpes at hand, foot and mouth, and the illness of most children with mild disease is self-limited and with good prognosis; but there are also some children who have severe complications of myocarditis and encephalitis, and the prognosis is worse[3]. Excessive activation of inflammatory response and immune response disorder are important characteristics in the development of HFMD. The changes in inflammatory response and immune response are particularly significant in children with severe HFMD. Nervous system is the most common involved area of severe HFMD children, and early assessment of the nerve damage degree in patients with HFMD is of positive value to improve prognosis of disease. NSE is a type of catalytic enzyme specifically expressed in the neurons, S100β is a calcium-binding protein specifically expressed in glial cells, and

✉Corresponding author: Qiao-Li Chai, Department of Pediatrics, Yulin Third Hospital in Shaanxi Province, Yulin City, Shaanxi Province, 719000.
Tel: 09123549356; 15319684478
Fund Project: Research Project of Shaanxi Health and Family Planning Commission No: 2016D076.

both NSE and S100β are used to assess the nerve injury in cerebral trauma, cerebral infarction, cerebral hemorrhage and other central nervous system diseases[4,5]. In the following study, the correlation of serum NSE and S100β levels with inflammatory response and immune response in children with HFMD complicated by encephalitis was analyzed.

2. Research subjects and research methods

2.1 General information of research subjects

Children diagnosed with hand-foot-mouth disease in Yulin Third Hospital between May 2015 and February 2017 were selected and divided into mild group and severe group based on the combination of central nervous system complications or not. Mild group (n=48) included 29 male cases and 19 female cases that were 3-10 years old; severe group (n=26) included 16 male cases and 10 female cases that were 3-9 years old. A total of 40 children who received physical examination in our hospital during the same period were selected as the control group, including 25 male cases and 15 female cases that were 4-11 years old. There was no significant difference in the general data of the three groups (P>0.05).

2.2 Serum index detection methods

5 mL of venous blood was collected from severe group and mild group on admission and before treatment, 5 mL of venous blood was collected from control group during physical examination, the blood was centrifuged to separate serum, and enzyme-linked immunosorbent assay kit was used to detect neuron-specific enolase (NSE), S100β protein (S100β), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), IL-10, IL-17 and procalcitonin (PCT) levels.

2.3 Peripheral blood immune cell detection

5 mL of venous blood was collected from severe group and mild group on admission and before treatment, 5 mL of venous blood was collected from control group during physical examination, fluorescent antibody of CD3, CD4, CD8, CD16, CD56, CD38 and HLA-DR were incubated, then Permeabilisation was added, the incubation continued, and finally flow cytometer was used to determine the contents of $CD3^+$T cells, $CD4^+$T cells, $CD8^+$T cells, NK cells, human leucocyte antigen -DR^+CD4^+ ($HLA\text{-}DR^+CD4^+$), $HLA\text{-}DR^+CD8^+$, $CD38^+CD4^+$ and $CD38^+CD8^+$.

2.4 Statistical methods

SPSS 19.0 software was used to input data, variance analysis was conducted and P<0.05 indicated statistical significance in differences in analysis results.

3. Results

3.1 Serum NSE and S100β levels

Analysis of serum NSE and S100β levels among three groups of subjects was as follows: serum NSE and S100β levels of severe group and mild group were significantly higher than those of control group, and serum NSE and S100β levels of severe group were significantly higher than those of mild group. Differences in pair-wise comparison of serum NSE and S100β levels were statistically significant among three groups of subjects (P<0.05).

Table 1.

Comparison of serum NSE and S100β levels among three groups of subjects (ng/mL).

Groups	n	NSE	S100β
Severe group	26	15.52±2.03*&	5.86±0.77*&
Mild group	48	9.45±1.15*	3.72±0.52*
Control group	40	5.63±0.78	2.15±0.29

*: compared with control group, P<0.05; &: compared with mild group, P<0.05.

3.2 Serum inflammatory mediator levels

Analysis of serum inflammatory mediators TNF-α (ng/mL), IL-6 (ng/mL), IL-10 (ng/mL), IL-17 (pg/mL) and PCT (ng/mL) levels among three groups of subjects was as follows: serum TNF-α, IL-6, IL-10, IL-17 and PCT levels of severe group and mild group were significantly higher than those of control group, and serum TNF-α, IL-6, IL-10, IL-17 and PCT levels of severe group were significantly higher than those of mild group. Differences in pair-wise comparison of serum TNF-α, IL-6, IL-10, IL-17 and PCT levels were statistically significant among three groups of subjects (P<0.05). Pearson correlation analysis showed that serum TNF-α, IL-6, IL-10, IL-17 and PCT levels in children with hand-foot-mouth disease were positively correlated with NSE and S100β levels.

Table 2.

Serum inflammatory mediator levels among in three groups of subjects.

Groups	n	TNF-α	IL-6	IL-10	IL-17	PCT
Severe group	26	218.65±37.28*&	97.28±10.25*&	164.56±20.34*&	48.65±6.82*&	9.52±1.15*&
Mild group	48	134.25±18.94*	65.41±8.24*	93.41±10.48*	32.15±4.57*	4.39±0.67*
Control group	40	56.75±7.873	22.51±3.85	36.58±5.63	16.28±2.03	0.89±0.11

*: compared with control group, P<0.05; &: compared with mild group, P<0.05.

3.3 Peripheral blood T cell subset and NK cell levels

Analysis of peripheral blood $CD3^+$T cell, $CD4^+$T cell, $CD8^+$T cell and NK cell levels among three groups of subjects was as follows: peripheral blood $CD3^+$T cell, $CD4^+$T cell, $CD8^+$T cell and NK cell levels of severe group and mild group were significantly lower than those of control group, and peripheral blood $CD3^+$T cell, $CD4^+$T cell, $CD8^+$T cell and NK cell levels of severe group were significantly lower than those of mild group. Differences in pair-wise comparison of peripheral blood $CD3^+$T cell, $CD4^+$T cell, $CD8^+$T cell and NK cell levels were statistically significant among three groups of subjects (P<0.05).

Table 3.

Peripheral blood T cell subset and NK cell levels in three groups of subjects.

Groups	n	$CD3^+$T cell	$CD4^+$T cell	$CD8^+$T cell	NK cell
Severe group	26	36.41±5.22$^{*\&}$	29.38±3.47$^{*\&}$	19.38±2.05$^{*\&}$	8.29±1.05$^{*\&}$
Mild group	48	48.51±6.35*	40.25±4.96*	24.51±3.48*	12.15±1.57*
Control group	40	64.91±8.58	48.59±6.22	32.15±4.85	16.28±2.16

*: compared with control group, P<0.05; $^{\&}$: compared with mild group, P<0.05.

3.4 Peripheral blood activated T lymphocyte subset levels

Analysis of peripheral blood activated T lymphocyte subsets $HLA\text{-}DR^+CD4^+$, $HLA\text{-}DR^+CD8^+$, $CD38^+CD4^+$ and $CD38^+CD8^+$ levels among three groups of subjects was as follows: peripheral blood $HLA\text{-}DR^+CD4^+$, $HLA\text{-}DR^+CD8^+$, $CD38^+CD4^+$ and $CD38^+CD8^+$levels of severe group and mild group were significantly higher than those of control group, and peripheral blood $HLA\text{-}DR^+CD4^+$, $HLA\text{-}DR^+CD8^+$, $CD38^+CD4^+$ and $CD38^+CD8^+$ levels of severe group were significantly higher than those of mild group. Differences in pair-wise comparison of peripheral blood $HLA\text{-}DR^+CD4^+$, $HLA\text{-}DR^+CD8^+$, $CD38^+CD4^+$ and $CD38^+CD8^+$ levels were statistically significant among three groups of subjects (P<0.05).

4. Discussion

NSE is a kind of catalytic enzyme specifically expressed in neurons, which is involved in the catalysis of glycolysis process, and has important value for the maintenance of the function of neurons[6]; S100β is a calcium-binding protein specifically expressed and secreted in glial cells, which regulates the integrity of the cytoskeleton structure, intracellular calcium homeostasis and the transduction of related signal pathway[7]. When trauma, infarction, bleeding and other factors cause the central nervous system damage, NSE and S100β in neurons and glial cells are released into the cerebrospinal fluid, and then enter into the blood circulation through the damaged blood brain barrier. Children with severe HFMD are mostly complicated by encephalitis, central nervous system is damaged[8,9], and the analysis of serum nerve injury marker molecules NSE and S100β levels in subjects in the study showed that serum levels of NSE and S100β in patients with HFMD were significantly higher than those in healthy volunteers and serum levels of NSE and S100β in severe group were significantly higher than those in mild group. This means that the course of hand-foot-mouth disease can cause neurologic injury, the risk of nerve function injury in children with severe hand-foot-mouth disease is larger than that in children with mild disease, and the degree of nerve function damage is also more significant than that in children with mild mild disease. The activation of inflammatory response is an important pathological change in the course of HFMD, and the synthesis and secretion of TNF-α, IL-6, IL-10, IL-17, PCT and other inflammatory mediators increased significantly[10]. TNF-α is an inflammatory mediator that changes in the early stage of inflammatory reaction, and it is secreted by activated mononuclear macrophages and has the biological function of promoting inflammatory reaction cascade amplification; IL-6 is a cytokine with inflammatory regulatory and immunoregulatory activity, which can promote the chemotaxis and infiltration of inflammatory cells in the process of inflammatory reaction; IL-10 is an inhibitory mediator, which can inhibit the over-activation of inflammatory response and be activated for compensation in the course of HFMD; IL-17 is a pro-inflammatory factor synthesized and secreted by Th17 cells, and it plays an important role in inflammatory injury of the central nervous system; PCT is the precursor substance of calcitonin, and in the pathologic state of inflammatory response activation, pro-inflammatory factors can induce the synthesis and secretion of PCT[11,12]. In the study, analysis of serum levels of inflammatory mediators in the subjects showed that the serum TNF-α, IL-6, IL-10, IL-17 and PCT levels in HFMD patients were significantly higher than those in healthy volunteers and serum TNF-α, IL-6, IL-10, IL-17 and PCT levels in severe group were significantly higher than those in mild group. This means that inflammatory response is significantly activated in the process of hand-foot-mouth disease, and the activation of inflammatory response in children with severe HFMD is more significant than that in children with mild HFMD. Further analysis of the correlation between inflammatory mediators and nerve injury markers showed that serum TNF-α, IL-6, IL-10, IL-17 and PCT levels in children with HFMD were positively correlated with NSE and S100β levels. This confirms that inflammatory response activation and inflammatory mediator release are the important

Table 4.

Peripheral blood activated T lymphocyte subset levels in three groups of subjects.

Groups	n	$HLA\text{-}DR^+CD4^+$	$HLA\text{-}DR^+CD8^+$	$CD38^+CD4^+$	$CD38^+CD8^+$
Severe group	26	30.25±4.85$^{*\&}$	24.26±3.75$^{*\&}$	9.67±1.16$^{*\&}$	59.63±7.25$^{*\&}$
Mild group	48	17.42±2.26*	18.97±2.32*	7.15±0.95*	44.29±6.72*
Control group	40	7.59±0.93	12.03±1.68	5.28±0.78	22.15±3.86

*: compared with control group, P<0.05; $^{\&}$: compared with mild group, P<0.05.

pathological links that cause the injury of nervous system in HFMD. Enterovirus infection is the main cause of HFMD, and hypoimmunity and poor antiviral capacity are the important reasons that make children become high risk group of HFMD. T lymphocytes and NK cells are important cell groups in the antiviral immune response process[13]. T cells become mature $CD3^+CD4^+$T cells and $CD3^+CD8^+$T cells after negative and positive selection, the former can be combined with histocompatibility complex II and activate B cells to kill virus through the humoral immune response, and the latter can be combined with the histocompatibility complex I to directly exert cytotoxic effect, and thereby neutralize the virus [14]. NK cells mainly mediate non-specific immune response and can kill virus through ADCC effect. In the study, analysis of the contents of peripheral blood immune cells in subjects showed that peripheral blood $CD3^+$T cell, $CD4^+$T cell, $CD8^+$T cell and NK cell levels of patients with HFMD were significantly lower than those of healthy volunteers and peripheral blood $CD3^+$T cell, $CD4^+$T cell, $CD8^+$T cell and NK cell levels of severe group were significantly lower than those of mild group and negatively correlated with serum NSE and S100β levels. This indicates that immune cell over-consumption and immune response inhibition can lead to the progression of HFMD and aggravate the nervous system injury.

HLA-DR and CD38 have important value for the activation of T lymphocytes in the process of antiviral immune response in T lymphocytes. HLA-DR can assist T lymphocytes to recognize the effective antigen composition of pathogens and make it activated; CD38 is a kind of single-stranded transmembrane glycoprotein on the cell surface, and is massively expressed in the activation and maturation of T lymphocytes[15]. In the study, analysis of the peripheral blood activated T lymphocyte levels in subjects showed that peripheral blood $HLA\text{-}DR^+CD4^+$, $HLA\text{-}DR^+CD8^+$, $CD38^+CD4^+$ and $CD38^+CD8^+$ levels of patients with HFMD were significantly higher than those of healthy subjects, and peripheral blood $HLA\text{-}DR^+CD4^+$, $HLA\text{-}DR^+CD8^+$, $CD38^+CD4^+$ and $CD38^+CD8^+$ levels of severe group were significantly higher than those of mild group. This means that T lymphocyte subsets are significantly activated in the progression of hand-foot-mouth disease, and the activated T cells will go through programmed death after completing the immune response, so the total number of T cells is decreasing in children with hand-foot-mouth disease. Further analysis of the correlation between activated T lymphocytes and nerve damage marker molecules showed that peripheral blood $HLA\text{-}DR^+CD4^+$, $HLA\text{-}DR^+CD8^+$, $CD38^+CD4^+$ and $CD38^+CD8^+$ levels in children with hand-foot-mouth disease were positively correlated with serum NSE and S100β levels. This confirms that the abnormal immune response is closely related to the injury of the nervous system in HFMD.

In the progress of HFMD, the risk of nerve injury increases significantly in children with severe disease, and the increase of serum NSE and S100β could early predict the occurrence of nerve injury; the excessive activation of inflammatory response, and the immune response disorder mediated by T cells and NK cells in the course of disease are the important pathological links of nerve injury in children with HFMD.

References

[1] Duong V, Mey C, Eloit M, Zhu H, Danet L, Huang Z, et al. Molecular epidemiology of human enterovirus 71 at the origin of an epidemic of fatal hand, foot and mouth disease cases in Cambodia. *Emerg Microbes Infect* 2016; **5**(9): e104.

[2] Huang Y, Zhou Y, Lu H, Yang H, Feng Q, Dai Y, et al. Characterization of severe hand, foot, and mouth disease in Shenzhen, China, 2009-2013. *J Med Virol* 2015; **87**(9): 1471-1479.

[3] Ma S, Zhang Y, Du C, Yang T, Liu Q, Pan Y, et al. Dynamic constitution of the pathogens inducing encephalitis in hand, foot and mouth disease in kunming, 2009-2011. *Jpn J Infect Dis* 2015; **68**(6): 504-510.

[4] Lai PM, Du R. Association between s100b levels and long-term outcome after aneurysmal subarachnoid hemorrhage: systematic review and pooled analysis. *PLoS One* 2016; **11**(3): e0151853.

[5] Li K, Jia J, Wang Z, Zhang S. Elevated serum levels of nse and s-100β correlate with increased risk of acute cerebral infarction in asian populations. *Med Sci Monit* 2015; **30**(21): 1879-1888.

[6] Thelin EP, Jeppsson E, Frostell A, Svensson M, Mondello S, Bellander BM, et al. Utility of neuron-specific enolase in traumatic brain injury; relations to S100B levels, outcome, and extracranial injury severity. *Crit Care* 2016; **8**(20): 285.

[7] Ye H, Wang L, Yang XK, Fan LP, Wang YG, Guo L. Serum S100B levels may be associated with cerebral infarction: a meta-analysis. *J Neurol Sci* 2015; **348**(1-2): 81-88.

[8] Sui M, Huang X, Li Y, Ma X, Zhang C, Li X, et al. Application and comparison of laboratory parameters for forecasting severe hand-foot-mouth disease using logistic regression, discriminant analysis and decision tree. *Clin Lab* 2016; **62**(6): 1023-1031.

[9] Ong KC, Wong KT. Understanding enterovirus 71 neuropathogenesis and its impact on other neurotropic enteroviruses. *Brain Pathol (Zurich, Switzerland)* 2015; **25**(5): 614-624.

[10]Duan G, Yang H, Shi L, Sun W, Sui M, Zhang R, et al. Serum inflammatory cytokine levels correlate with hand-foot-mouth disease severity: a nested serial case-control study. *PLoS One* 2014; **9**(11): e112676.

[11]Zhang SY, Xu MY, Xu HM, Li XJ, Ding SJ, Wang XJ, et al. Immunologic characterization of cytokine responses to enterovirus 71 and coxsackievirus a16 infection in children. *Medicine (Baltimore)* 2015; **94**(27): e1137.

[12]Shao P, Wu X, Li H, Wu Z, Yang Z, Yao H. Clinical significance of inflammatory cytokine and chemokine expression in hand, foot and mouth disease. *Mol Med Rep* 2017; **15**(5): 2859-2866.

[13]Zhou JF, Chen ZY, Yang SM, Chen JZ, Zhou LY, Wang YF, et al. Clinical features and peripheral blood T lymphocyte subsets in hand, foot, and mouth disease according to different pathogens. *Indian J Pediatr* 2017; **84**(2): 124-127.

[14]Bengs S, Marttila J, Susi P, Ilonen J. Elicitation of T-cell responses by structural and non-structural proteins of coxsackievirus B4. *J Gen Virol* 2015; **96**(Pt 2): 322-330.

[15]N'Guyen Y, Lesaffre F, Metz D, Tassan S, Saade Y, Boulagnon C, et al. Enterovirus but not Parvovirus B19 is associated with idiopathic dilated cardiomyopathy and endomyocardial CD3, CD68, or HLA-DR expression. *J Med Virol* 2017; **89**(1): 55-63.