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Lymphocyte subset contents in cerebrospinal fluid of children with viral encephalitis

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ABSTRACT

Objective: To study the lymphocyte subset contents in cerebrospinal fluid of children with viral encephalitis and their correlation with disease. **Methods:** Children with viral encephalitis were selected as VE group, children excluded of central nervous system infection by lumbar puncture or children without central nervous system diseases but receiving surgery with spinal anesthesia were selected as control group, and then cerebrospinal fluid and serum were collected to detect lymphocyte subset contents, nerve injury molecule contents as well as inflammatory response indicators and oxidative stress response indicators. **Results:** $CD3^+$, $CD3^+CD4^+$, CD4/CD8 and $CD16^+CD56^+$ in cerebrospinal fluid of VE group were lower than those of control group, and both $CD3^+CD8^+$ and $CD19^+$ were higher than those of control group; $CD3^+$, $CD3^+CD4^+$, CD4/CD8 and $CD16^+CD56^+$ in cerebrospinal fluid of children with abnormal MRI were lower than those of children with normal MRI, and both $CD3^+CD8^+$ and $CD19^+$ were higher than those of children with normal MRI; NSE, MBP, S-100 and NPT contents in cerebrospinal fluid and serum of VE group were significantly higher than those of control group and had good correlation with lymphocyte subset contents; MMP9, TNF-α and IL-6 contents in cerebrospinal fluid of VE group were significantly higher than those of control group, and SOD and GSH-Px contents were significantly lower than those of control group and had good correlation with lymphocyte subset contents. **Conclusions:** $CD4^+/CD8^+$T lymphocyte ratio and NK cell content decrease, and B lymphocyte content increases in cerebrospinal fluid of children with viral encephalitis, and lymphocyte subset contents have inhibitory effect on MRI manifestation, degree of inflammatory response and oxidative stress response.

1. Introduction

Viral encephalitis (VE) is a common pediatric infectious disease of the central nervous system that mostly occurs in preschool children. That virus infects central nervous system can lead to neuron and glial cell damage and cause headache, fever, vomiting and meningeal irritation and other symptoms. Early symptoms of VE are mostly not typical, clinical missed diagnosis rate and misdiagnosis rate are high, light cases can leave sequela of nerve function injury and severe cases may threaten life safety[1,2]. Therefore, exploring characteristic changes in nervous system after virus infection and looking for molecules with diagnostic value and condition assessment value received much attention. Studies believe that virus infection can cause the body's immune response to the virus antigen, manifested as changes of a variety of immune cell components in the nervous system[3,4]. Lymphocytes are the important cells mediating immune response, and virus infection can cause abnormal contents of a variety of lymphocytes. In the following research, the lymphocyte subset contents in cerebrospinal fluid of children with viral encephalitis and their correlation with the illness were analyzed.

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2. Materials and methods

2.1. Subjects

A total of 30 children with viral encephalitis were selected as VE group, and all of them met the following criteria: (1) acute or subacute onset; (2) with the symptoms or signs of brain parenchyma or meninges damage, such as convulsion, hemiplegia, rigidity of the neck and nape and so on; (3) with one of the following circumstances: a) respiratory traction infection or diarrhea 1 week before the disease, and fever or mumps when admitted; b) consistent with changes of viral encephalitis by CSF conventional and biochemical examination; c) abnormal EEG finding, cranial CT or MRI showing signs of inflammation of the brain. 30 cases of children excluded of central nervous system infection by lumbar puncture or children without central nervous system diseases but receiving surgery with spinal anesthesia were selected as control group. Comparison of general information between two groups showed no differences.

2.2.Sample collection

A total of 2 mL CSF was collected through lumbar puncture as required from the included cases on the day of admission or the next day, and 2 mL CSF was collected during lumbar puncture.

2.3.Sample detection

2.3.1. Flow cytometry

MultiTEST kits were used for detection, 50 μL cerebrospinal fluid sample was added in tubes with different CD molecule fluorescent antibodies and incubated away from light for 15 min, 450 μL hemolysin was added, then the sample was continued to be incubated for 15 min, and then $CD3^+$, $CD3^+CD4^+$, $CD16^+CD56^+$, $CD3^+CD8^+$ and $CD19^+$ contents were detected in flow cytometer and calculated.

2.3.2. Enzyme-linked immunosorbent assay

Elisa kits were used to detect NSE, MBP, S-100, NPT, MMP9, TNF-α, IL-6, SOD and GSH-Px contents in serum and cerebrospinal fluid.

2.4. Statistical methods

SPSS21.0 software was used to input and analyze data, analysis of measurement data was by *t* test and differences were considered to be statistically significant at a level of $P<0.05$.

3.Results

3.1. Lymphocyte contents in cerebrospinal fluid

$CD3^+$, $CD3^+CD4^+$, CD4/CD8 and $CD16^+CD56^+$ in cerebrospinal fluid of VE group were lower than those of control group, and $CD3^+CD8$+ and $CD19^+$ were higher than those of control group (Table 1).

3.2. Lymphocyte contents in cerebrospinal fluid of VE children with different MRI characteristics

$CD3^+$, $CD3^+CD4^+$, CD4/CD8 and $CD16^+CD56^+$ in cerebrospinal fluid of children with abnormal MRI were lower than those of children with normal MRI, and both $CD3^+CD8^+$ and $CD19^+$ were higher than those of children with normal MRI (Table 2).

3.3. Nerve injury molecule contents in cerebrospinal fluid and serum

NSE, MBP, S-100 and NPT contents in cerebrospinal fluid of VE group were significantly higher than those of control group; NSE, MBP, S-100 and NPT contents in serum of VE group were significantly higher than those of control group (Table 3).

3.4. Inflammatory response and oxidative stress response indicators

MMP9, TNF-α and IL-6 contents in cerebrospinal fluid of VE group were significantly higher than those of control group, and SOD and GSH-Px contents were significantly lower than those of control group (Table 4).

4. Discussion

Viral encephalitis is a common infectious disease of the central nervous system in preschool children. Virus infection may result in the immune response change in nervous system, and the main manifestation of children with viral encephalitis is the immune response change in central nervous system[5]. T lymphocytes, B lymphocytes and NK cells are the key cells mediating cellular

Table 1

Lymphocyte contents in cerebrospinal fluid.

Groups	$CD3^+$ (%)	$CD3^+CD4^+$ (%)	$CD3^+CD8^+$ (%)	CD4/CD8	$CD19^+$ (%)	$CD16^+CD56^+$ (%)
VE group	60.3±7.4	32.5±4.1	30.3±3.8	1.08±0.12	27.1±3.1	7.9±0.8
Control group	72.5±8.1	40.3±4.9	25.9±2.9	1.56±0.17	16.4±2.0	12.1±1.4
t	5.474	5.912	5.785		7.283	6.586
P	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05

Table 2

Lymphocyte contents in cerebrospinal fluid of VE children with different MRI characteristics.

Groups	$CD3^+$	$CD3^+CD4^+$	$CD3^+CD8^+$	CD4/CD8	$CD19^+$	$CD16^+CD56^+$
AbnormalMRI	53.9±6.9	27.3±3.5	34.8±4.3	0.78±0.09	33.2±3.8	5.4±0.6
Normal MRI	66.6±7.8	36.4±4.4	27.7±3.2	1.31±0.15	22.9±2.2	10.2±1.2
t	6.122	5.887	5.373	9.382	6.328	9.782
P	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05

Table 3

Nerve injury molecule contents in cerebrospinal fluid and serum.

Indexes	VE group	Control group	*t*	*P*
Serum				
NSE (μg/L)	20.35±2.58	11.47±1.26	9.182	< 0.05
MBP (μg/L)	0.73±0.09	0.34±0.04	12.485	< 0.05
S-100 (μg/L)	1.06±0.14	0.64±0.08	7.659	< 0.05
NPT (nmol/L)	22.34±2.57	7.65±0.78	19.393	< 0.05
Cerebrospinal fluid				
NSE	10.34±1.25	4.58±0.54	12.319	< 0.05
MBP	0.93±0.10	0.45±0.06	11.337	< 0.05
S-100	1.27±0.14	0.70±0.08	7.689	< 0.05
NPT (nmol/L)	34.85±4.95	11.34±1.64	22.128	< 0.05

Table 4

Inflammatory response and oxidative stress response indicator contents in cerebrospinal fluid.

Indexes	VE group	Control group	*t*	*P*
Cerebrospinal fluid				
MMP9 (μg/L)	0.72±0.09	0.28±0.03	17.572	< 0.05
TNF-α (μg/L)	0.41±0.05	0.23±0.02	7.494	< 0.05
IL-6 (μg/L)	0.57±0.07	0.19±0.02	20.484	< 0.05
SOD (U/mL)	18.57±2.15	34.29±4.18	8.954	< 0.05
GSH-Px (U/mL)	11.39±1.47	25.46±2.88	13.285	< 0.05

immunity, humoral immunity and innate immunity[6,7]. CD3 is a marker molecule of mature T cells and $CD3^+$ positive rate can reflect the overall level of cellular immunity. CD4 is a marker molecule of helper T lymphocytes, CD8 is a marker molecule of suppressor T lymphocytes, and interaction and inhibition between the two is the key to the regulation of cellular immune response; the positive rate of $CD3^+CD4^+$ and $CD3^+CD8^+$ as well as $CD4^+/CD8^+$ ratio can reflect the activation or inhibition state of cellular immunity[8]. CD19 is a marker molecule of B lymphocyte surface, and activated B cells can synthesize and secrete immunoglobulin so as to neutralize viruses and toxins[9]. CD16 and CD56 are marker molecules of NK cell surface, and NK cells belong to innate immune cells, participate in the composition of nonspecific immune system and can play an antiviral role in the early stage of the virus infection[10].

In order to clarify the characteristics of immune response in central nervous system in children with viral encephalitis, the contents of different lymphocyte subsets in cerebrospinal fluid were detected and analyzed, and results showed that analysis of lymphocyte contents in cerebrospinal fluid of VE group and control group was as follows: $CD3^+$, $CD3^+CD4^+$, CD4/CD8 and $CD16^+CD56^+$ in cerebrospinal fluid of VE group were lower than those of control group, and $CD3^+CD8^+$ and $CD19^+$ were higher than those of control group. Further analysis of above lymphocyte subset contents of VE children with different MRI characteristics showed that the change of above lymphocyte subset contents of children with abnormal MRI was more obvious than that of children with normal MRI. It indicated that in cases of viral encephalitis, the characteristics of immune response were as follows: (1) cellular immune was in disorder, helper T cell content decreased and suppressor T cells increased; (2) B lymphocyte activation increased, and specific antibody increased and neutralized the viruses and toxins; (3) NK cells were largely consumed in early viral infections, and the content significantly reduced.

That virus infects the nervous system will cause extensive damage of brain parenchyma, and the pathological characteristics are brain edema, perivascular inflammatory cell infiltration as well as nerve cell degeneration, damage and necrosis. There is rich expression of NSE, MBP, NPT in central nervous system and in cases of nerve tissue injury, above molecules are released into cerebrospinal fluid, and then enter bloodstream through the blood-brain barrier[11,12]. NSE is the key enzyme in neurons that catalyzes the process of glycolysis, and it participates in the regulation of energy metabolism in neurons; MBP is a kind of protein specifically expressed on myelin membrane, and it is nerve tissue-specific; S-100B is a small molecular weight-protein expressed in glial cells and neurons, and it can regulate cell growth and signal transduction; NPT is a cytokine

secreted by mononuclear macrophages and dendritic cells in central nervous system, and inflammatory response can stimulate the expression and generation of NPT. Analysis of nerve injury molecule contents in cerebrospinal fluid and serum showed that NSE, MBP, S-100 and NPT contents in cerebrospinal fluid and serum of VE group were significantly higher than those of control group.

In the development of viral encephalitis, inflammatory response and oxidative stress response are important ways causing nerve damage. Inflammatory response is mainly mediated by a variety of inflammatory cytokines, and MMP9, TNF-α, IL – 6 and so on are directly related to neuron damage. MMP9 is a proteolytic enzyme that is produced by neurons and glial cells and can destroy the stability of blood-brain barrier; IL-6 is a kind of multifunctional cytokine that can recruit inflammatory cells in local nervous tissue and amplify inflammatory reaction, and can also stimulate B cell proliferation and activation and increase antibody generation; TNF-α is produced by activated macrophages, and it is the most important inflammatory mediator mediating inflammatory reaction and causing inflammatory tissue damage[13,14]. Oxidative stress is mainly mediated by locally excessively produced oxygen free radicals, and it can cause large consumption of antioxidants SOD and GSH-Px[15]. Analysis of inflammatory response and oxidative stress response-related molecule contents showed that MMP9, TNF-α and IL-6 contents in cerebrospinal fluid of VE group were significantly higher than those of control group, and SOD and GSH-Px contents were significantly lower than those of control group.

To sum up, $CD4^+$/$CD8^+$T lymphocyte ratio and NK cell content decrease, and B lymphocyte content increases in cerebrospinal fluid of children with viral encephalitis, and lymphocyte subset contents have inhibitory effect on MRI manifestation, degree of inflammatory response and oxidative stress response.

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