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Original Article

Predictive value of procalcitonin, tumor necrosis factor-α and neuron-specific enolase levels in cerebrospinal fluid and serum for viral encephalitis severity and prognosis

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Abstract: Objective: To investigate the relationship between the levels of procalcitonin (PCT), tumor necrosis factor-α (TNF-α) and neuron-specific enolase (NSE) in cerebrospinal fluid and serum, as well as the severity of viral encephalitis and their predictive value for prognosis. Methods: This was a retrospective study. Eighty-four patients with viral encephalitis were enrolled in the observation group, and 50 healthy children were included as the control group. The levels of PCT, TNF-α and NSE in cerebrospinal fluid and serum were detected at the time of hospital admission and disease recovery. Results: The levels of PCT, TNF-α and NSE in the cerebrospinal fluid and serum of patients in the observation group were higher than those in the healthy control group (P<0.001). The levels of PCT, TNF-α and NSE in the cerebrospinal fluid and serum of patients with severe viral encephalitis were higher than those in the mild group (P<0.001). The levels of PCT, TNF-α and NSE in cerebrospinal fluid and serum of patients with viral encephalitis in acute phase were higher than those in recovery group (P<0.001). The levels of PCT, TNF-α and NSE in cerebrospinal fluid and serum of patients with poor prognosis were higher than those with good prognosis (P<0.001). Conclusion: The detection of cerebrospinal fluid and serum levels of PCT, TNF-α and NSE in patients with viral encephalitis has an important clinical value for the severity of disease and prognosis assessment, and it is worthy of clinical application.

Keywords: Viral encephalitis, procalcitonin, tumor necrosis factor-α, neuron-specific enolase, prognosis

Introduction

Viral encephalitis is a common central nervous system disease in pediatrics. The onset is acute and the clinical manifestations are different [1, 2]. It is mainly caused by the infection of virions in the brain parenchymal area [3]. Viral infections are contagious and can form epidemic outbreaks in areas with great risk [4, 5]. Viral encephalitis has a high clinical mortality rate and disability rate. Most of the mild patients have a good prognosis, and 80%-95% of which can survive. However, the disability rate is as high as 20% in severe patients, and the incidence of sequelae is 50-70% in the literature; severe patients have poor disease control and poor prognosis, which imposes a heavy burden on the family and social medical resources [6, 7]. Therefore, it is of great significance to judge early the severity and prognosis of the disease.

Procalcitonin (PCT) is an indicator of the severity of infection [8]. It is also sensitive to viral infections and can rapidly increase after infection in the body. A study has found that an appropriate amount of PCT can stimulate the body's immune proteins to play an anti-infective role, but its excessive expression can cause inflammatory reactions and damage to the body [9]. In addition, when the central nervous system is infected, the infected pathogen not only damages the brain tissue through the blood-brain barrier, but also directly stimulates

the expression of tumor necrosis factor-α (TNF-α) which is one of the pro-inflammatory factors [10]. Early nerve cell injury can stimulate the expression of neuron-specific enolase (NSE), which can be used as one of the sensitive indicators for clinical cell damage [11]. Therefore, this study chose patients with viral encephalitis as research subjects to investigate the predictive value of cerebrospinal fluid and serum PCT, TNF-α and NSE levels for disease severity and prognosis.

Materials and methods

Clinical data

In this retrospective study, a total of 84 patients with viral encephalitis who were admitted to Xingtai People's Hospital from January 2017 to April 2019 were enrolled in the observational group, including 48 males and 36 females, aged 2-11 years, and 50 healthy children were selected as the control group, including 27 males and 23 females, aged 2-12 years. All enrolled patients or their guardians signed the informed consent. The study was approved by the Ethics Committee of Xingtai People's Hospital.

Inclusion and exclusion criteria

Inclusion criteria: Patients who met the diagnosis of viral encephalitis in the 2010 edition of "Zhufutang Practical Pediatrics" [12]; patients aged 1-15 years; patients diagnosed with viral encephalitis for the first time; patients with complete clinical data.

Exclusion criteria: Patients with severe malnutrition, or tumors, etc.; patients with suppurative meningitis or purulent meningitis; patients combined with other infections.

Grouping

Patients were grouped according to the diagnosis of viral encephalitis. According to the severity of the disease, the patients were divided into 50 patients in the mild group and 34 patients in the severe group. According to the stage of disease, the patients were divided into 84 patients in acute phase and 84 patients in recovery period. According to the evaluation criteria of post-treatment efficacy [13], the patients were divided into 58 patients with good prognosis and 26 patients with poor prognosis. Mild patients: Children with non-specific symptoms such as fever, dizziness, abdominal pain, and respiratory symptoms. Severe patients: Children with disorientation and coma. Acute phase: The child's condition often lasted for several days to 2-3 weeks. Recovery period: The child's condition lasted for several weeks to several months, manifesting as difficulty in concentration, tremor, fatigue and personality change. Good prognosis: Patients recovered well after treatment, without neurological sequelae. Poor prognosis: Neurological symptoms left after treatment.

Methods

Determination of PCT, TNF-α, and NSE levels: Two tubes of 5 mL of venous blood were collected from each patient at admission and during disease recovery, and cerebrospinal fluid was collected. The collected blood samples were stored in a sterile tube of ethylenediaminetetra acetic acid, stored in a refrigerator at 4°C for 15 minutes, and then centrifuged at 3,300 rpm to separate serum and plasma. The separated plasma was added to a phosphate buffer solution containing 40 μL of protease inhibitor and stored in a freezer at -80°C. Based on the automatic multi-function microplate reader (Thermo Company, USA), the PCT and NSE levels were determined by enzyme-linked immunosorbent assay (ELISA) (Shanghai Jierui Bioengineering Co., Ltd., China), and the immunoturbidimetry method (Shanghai Jierui Bioengineering Co., Ltd., China) was adopted to determine serum TNF-α levels. The cerebrospinal fluid was taken from the lumbar spinal cord and stored at -70°C; the levels of PCT, TNF-α and NSE were determined by ELISA. The specific operation steps were strictly in accordance with the instructions.

Statistical analysis

SPSS 17.0 software (Asia Analytics Formerly SPSS, China) was used for statistical analysis. The continuous variables obeying normal distribution by the Kolmogorov test were expressed as mean ± standard deviation ($\bar{x}$ ± sd). Those with homogeneity of variance were tested by independent sample t test, denoted by t. Data that did not obey the normal distribution and the homogeneity of the variance were analyzed by the rank sum test, denoted by Z. The enumeration data were compared by χ^2 test, denot-

Table 1. Comparison of general data between observation group and control group

	Observation (n=84)	Control (n=50)	χ^2/t	P
Gender (male:female)	48:36	27:23	0.126	0.723
Age (years)	6.3±1.6	6.5±1.6	0.799	0.426

Table 2. Comparison of general and baseline data of patients with mild and severe disease

	Mild group (n=50)	Severe group (n=34)	χ^2/t	P
Gender (male:female)	28:22	20:14	0.066	0.797
Age (years)	6.3±1.6	6.3±1.7	0.071	0.944
Virus infection			0.256	0.992
Rotavirus	14	10		
Human astrovirus	12	7		
Human cup virus	11	8		
Intestinal adenovirus	9	6		
Echovirus	2	1		
Other	2	2		
Time of onset (hours)	21.00±8.90	22.03±10.43	0.485	0.629
Pediatric critical score	67.98±3.98	74.68±5.66	6.369	<0.001
Glasgow coma score at admission	6.14±1.41	8.12±1.93	5.413	<0.001

ed by χ^2. P<0.05 was considered statistically significant.

Results

Clinical baseline data

There was no significant difference in gender and age between the observation group and the control group (P>0.05). There were no significant differences in gender, age, virus infection and onset time between the mild and severe patients (P>0.05), but the pediatric critical score and Glasgow coma index of patients in the severe group were higher than those in the mild group (P<0.05). There were no significant differences in gender, age, virus infection and onset time among patients with different prognosis (P>0.05), but the scores of pediatric criticality and the Glasgow coma of patients with poor prognosis were higher than those with good prognosis (P<0.05). See **Tables 1-3**.

Comparison of PCT, TNF-α and NSE levels between patients with viral encephalitis and control group

The levels of PCT, TNF-α and NSE in cerebrospinal fluid and serum of patients with viral encephalitis were significantly higher than those in the control group (P<0.05). See **Table 4** and **Figure 1**.

Comparison of PCT, TNF-α and NSE levels in cerebrospinal fluid and serum of patients with mild and severe viral encephalitis

The levels of PCT, TNF-α and NSE in cerebrospinal fluid and serum of patients with severe viral encephalitis were significantly higher than those in the mild group (P<0.05). See **Table 5**.

Comparison of PCT, TNF-α and NSE levels in cerebrospinal fluid and serum of patients in acute and recovery period

The levels of PCT, TNF-α and NSE in cerebrospinal fluid and serum of patients with viral encephalitis in acute phase were significantly higher than those in the recovery group (P<0.05). See **Table 6**.

Comparison of PCT, TNF-α and NSE levels in cerebrospinal fluid and serum of patients with different prognosis at admission

The levels of PCT, TNF-α and NSE in cerebrospinal fluid and serum of patients with poor prognosis were significantly higher than those with good prognosis (P<0.05). See **Table 7**.

Discussion

Previous studies of patients with viral encephalitis have found that patients often have symptoms of viral diarrhea in the early stage of the disease. The infected viruses are mainly human astrovirus, cup virus, intestinal adenovirus and echovirus that can cause outbreaks [14, 15]. In this study, patients with viral encephalitis were also found to be infected with the above viruses. The clinical manifestations of viral encephalitis mainly include hyperemia of brain paren-

Table 3. Comparison of general data of patients with different prognosis

	Good prognosis (n=58)	Poor prognosis (n=26)	χ^2/t	P
Gender (male:female)	32:26	16:10	0.297	0.586
Age (years)	6.4±1.5	6.2±2.0	0.438	0.663
Virus infection			0.957	0.966
Rotavirus	17	7		
Human astrovirus	13	6		
Human cup virus	14	5		
Intestinal adenovirus	10	5		
Echovirus	2	1		
Other	2	2		
Time of onset (hours)	21.38±8.38	21.50±11.82	0.053	0.957
Pediatric critical score	69.07±4.63	74.31±6.40	4.238	<0.001
Glasgow coma score at admission	6.43±1.54	8.07±2.15	3.977	<0.001

Table 4. Comparison of PCT, TNF-α and NSE levels in cerebrospinal fluid and serum

Group	Number of patients	PCT (ng/mL)		TNF-α (pg/L)		NSE (μg/L)	
		Serum	Cerebrospinal fluid	Serum	Cerebrospinal fluid	Serum	Cerebrospinal fluid
Observation	84	0.43±0.15	0.38±0.16	43.05±23.98	19.83±9.51	16.80±12.38	15.94±3.28
Control	50	0.14±0.10	0.12±0.10	8.34±2.62	4.82±1.24	6.15±3.42	5.02±2.79
t		13.488	11.808	13.134	14.265	7.420	20.500
P		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Note: PCT: procalcitonin; TNF-α: tumor necrosis factor-α; NSE: neuron-specific enolase.

chyma, edema of brain tissue, inflammatory infiltration around the blood vessels, and necrosis of nerve cells. If the condition is delayed or poorly controlled, it may cause irreversible damage to the nerves, and even endangers the life of patients [16]. Early clinical evaluation and active intervention have positive implications for the prognosis of patients.

Clinical studies of PCT have found that PCT has begun to rise at an earlier stage of infection and is more sensitive than other inflammatory factors [17], and PCT levels are positively correlated with the severity of infection [18]. PCT not only increases significantly in bacterial infections, but also increases in viral infections [8]. PCT is extremely low in serum and cerebrospinal fluid of healthy patients, generally below 0.05 ng/mL. However, after 2 hours of infection in the body, PCT begins to rise, peaks at 12-24 hours and is not interfered with by hormones and antibiotics. It suggests that PCT is important for differential diagnosis, evaluation of efficacy, and prognosis of disease [19, 20]. The study also found that the PCT level in serum and cerebrospinal fluid of children with viral encephalitis was significantly higher than that of healthy control group, almost 3 times higher than the healthy control group. Further analysis of the children according to severity, period and prognosis found that the expression of PCT in cerebrospinal fluid and serum of the severe children was about 1.47 times than those of the mild children. The PCT level in serum and cerebrospinal fluid of children in acute phase was 1.8 times than those of patients in recovery period. The PCT levels in serum and cerebrospinal fluid of children with poor prognosis were 1.5 times and 1.6 times higher than those with good prognosis, respectively.

A study has found that inflammatory factors are involved in all stages of viral encephalitis [21]. TNF-α has a dual biological effect, which plays a role in the regulation of anti-infection and tissue repair inflammatory response at low levels, but when its level is high, it will destroy the body's immunity, activate neutrophils, and stimulate the secretion of inflammatory factors, resulting in increased vascular permeability

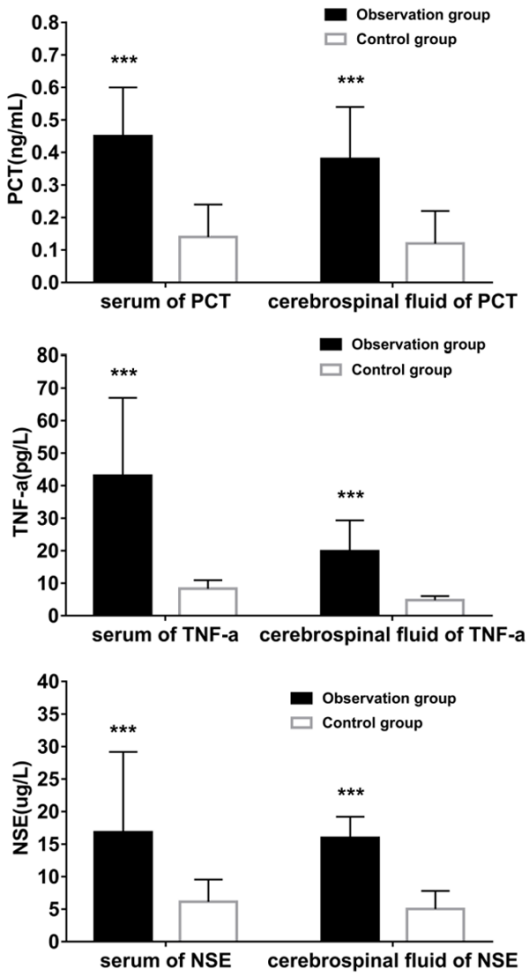

Figure 1. Comparison of PCT, TNF-α and NSE levels in cerebrospinal fluid and serum. Compared with the control group, ***P<0.001. PCT: procalcitonin; TNF-α: tumor necrosis factor-α; NSE: neuron-specific enolase.

[22]. In the study of patients with viral encephalitis, it was found that the mechanism of TNF-α production is complicated, mainly because the virus stimulates the secretion of interleukin-1 by glial cells, which in turn produces TNF-α [23]. TNF-α produces cytotoxicity locally and enhances the biological effects of endotoxin, resulting in direct and indirect damage to brain tissue [24]. Some studies have found that TNF-α is significantly elevated in cerebrospinal fluid of patients with viral encephalitis in acute phase [25]. This study found that serum and cerebrospinal fluid TNF-α levels in children with viral encephalitis were higher than those in healthy control group. The serum and cerebrospinal fluid TNF-α levels were 5.2 and 5.1 times higher than those of the healthy control group, respectively. Further studies found that serum and cerebrospinal fluid TNF-α levels of severe patients were 2.6 and 2.4 times higher than those of mild patients, respectively. The serum and cerebrospinal fluid TNF-α levels of children in acute phase were 2.6 and 2.4 times higher than those in the recovery period. Serum and cerebrospinal fluid TNF-α levels of children with poor prognosis were 2.1 and 1.8 times higher than those of children with good prognosis. This suggested that TNF-α was active in severe children, acute phase and children with poor prognosis, which was associated with the phase, severity and prognosis of the disease.

Neuron-specific enolase (NSE) is a specific enzyme for glycolysis. After 8 hours of brain tissue injury, NSE in serum and cerebrospinal fluid can rise to a peak [26]. Brain diseases cause brain tissue damage, making the permeability of the blood-brain barrier change, and NSE can enter the bloodstream, causing a significant increase in NSE levels in the blood [27]. The most common clinical symptom of patients with viral encephalitis is nerve damage. Therefore, the detection of NSE levels in the serum and cerebrospinal fluid of patients in the early stage of the disease can quickly determine whether the brain tissue is damaged and the extent of the damage. This study found that the levels of NSE in serum and cerebrospinal fluid in children with viral encephalitis were 2.7 and 3.2 times higher than those in healthy control group, respectively. Further studies found that the serum and cerebrospinal fluid NSE levels in severe patients were 3.6 and 2.4 times higher than those in mild patients, and the serum and cerebrospinal fluid NSE levels of patients in acute phase were 2.3 and 1.8 times higher than those in recovery period, respectively. The serum and cerebrospinal fluid NSE levels in children with poor prognosis were 2.7 and 1.3 times higher than those with good prognosis. This suggested that there might be a correlation between NSE levels and severe brain damage in severe children, acute phase and children with poor prognosis.

This study had some limitations. The sample size of this study was small, so it is necessary to expand the sample size for future research. The observation time was short, and there were many external influencing factors, such as dif-

Table 5. Comparison of PCT, TNF-α and NSE levels in cerebrospinal fluid and serum of patients with mild and severe viral encephalitis

Group	Number of patients	PCT (ng/mL)		TNF-α (pg/L)		NSE (μg/L)	
		Serum	Cerebrospinal fluid	Serum	Cerebrospinal fluid	Serum	Cerebrospinal fluid
Severe	34	0.51±0.11	0.47±0.15	68.24±15.67	30.14±4.24	29.38±9.12	18.44±2.42
Mild	50	0.37±0.14	0.32±0.13	25.92±8.30	12.82±4.14	8.24±4.35	14.23±2.64
t		5.283	4.727	14.428	18.551	12.580	7.401
P		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Note: PCT: procalcitonin; TNF-α: tumor necrosis factor-α; NSE: neuron-specific enolase.

Table 6. Comparison of PCT, TNF-α and NSE levels in cerebrospinal fluid and serum of patients in acute and recovery period

Group	Number of patients	PCT (ng/mL)		TNF-α (pg/L)		NSE (μg/L)	
		Serum	Cerebrospinal fluid	Serum	Cerebrospinal fluid	Serum	Cerebrospinal fluid
Acute phase	84	0.43±0.15	0.38±0.16	43.05±23.98	19.83±9.51	16.80±12.38	15.94±3.28
Recovery period	84	0.24±0.17	0.21±0.16	16.64±11.47	8.26±5.03	7.22±4.01	8.88±5.44
t		7.270	7.237	14.428	18.551	12.580	7.401
P		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Note: PCT: procalcitonin; TNF-α: tumor necrosis factor-α; NSE: neuron-specific enolase.

Table 7. Comparison of PCT, TNF-α and NSE levels in cerebrospinal fluid and serum of patients at admission

Group	Number of patients	PCT (ng/mL)		TNF-α (pg/L)		NSE (μg/L)	
		Serum	Cerebrospinal fluid	Serum	Cerebrospinal fluid	Serum	Cerebrospinal fluid
Poor prognosis	26	0.55±0.14	0.52±0.16	66.94±21.31	28.96±8.12	29.46±12.10	18.89±3.01
Good prognosis	58	0.38±0.12	0.32±0.11	32.34±16.16	15.74±6.91	11.12±7.27	14.62±2.44
t		5.807	5.712	8.195	7.671	7.170	6.887
P		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Note: PCT: procalcitonin; TNF-α: tumor necrosis factor-α; NSE: neuron-specific enolase.

ferent treatment options, differences in visit time, and recovery time of the disease. Therefore, it is necessary to increase the follow-up time for firther research.

In summary, the detection of cerebrospinal fluid and serum PCT, TNF-α and NSE levels in patients with viral encephalitis has important clinical value for the assessment of the severity and prognosis of the disease and is worthy of clinical application.

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Disclosure of conflict of interest

None.

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