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MRI changes and expressions of neuron-specific enolase and monocyte chemoattractant protein-1 in cerebrospinal fluid in patients with severe herpes simplex virus encephalitis

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ABSTRACT

The objective of this research was to analyze the MRI changes and the expression of neuron-specific enolase (NSE) and monocyte chemoattractant protein-1 (MCP-1) in cerebrospinal fluid (CSF) of patients with severe herpes simplex encephalitis. For this purpose, 68 patients with severe herpes simplex virus encephalitis diagnosed and treated in our hospital from April 2020 to April 2021 were selected as the study objects of the study group. In addition, 68 healthy people who underwent normal physical examinations in our hospital were selected as the control group at the same time. They were examined by magnetic resonance imaging (MRI) within one week after the study group was enrolled. CSF samples were collected one week after the onset of the disease in the study group and 2-4 days after the first spinal anesthesia in the control group, Enzyme linked immunosorbent assay (ELIEA) was used to detect the expression of NSE and MCP-1 in cerebrospinal fluid of the two groups, and the linear correlation between NSE and MCP-1 were analyzed. Results showed that compared with the control group, the expression of NSE and MCP-1 in the cerebrospinal fluid of the study group increased significantly ($P<0.05$). The expression of NSE and MCP-1 in patients with severe herpes simplex encephalitis in a coma was significantly higher than that in patients without severe herpes simplex encephalitis in a coma ($P<0.05$). NSE and MCP-1 were positively correlated ($r=0.597$, $P=0.001$). NSE and MCP-1 were risk factors for severe herpes simplex encephalitis, and the difference was statistically significant ($P<0.05$). In conclusion, magnetic resonance imaging of patients with severe herpes simplex encephalitis is characterized by multiple lesions in the temporal lobe, insula, and frontal lobe base (especially the marginal system involved) with unilateral or bilateral asymmetric distribution, and abnormal high expression of NSE and MCP-1 in the cerebrospinal fluid of such patients, which has important value in the early diagnosis of this disease.

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Introduction

Herpes simplex virus encephalitis is an acute virus infectious condition that occurs in the central nervous system (CNS), where the virus is mainly transmitted through sexual transmission, close contact and droplets. It is chiefly characterized by brain tissue hemorrhage, edema, necrosis and even worse clinical signs among severe patients (1-2). It has been indicated(3) that the recent application of acyclovir, a specific antiviral drug, in the treatment of viral encephalitis has dropped the mortality rate to about 20%. However, early diagnosis can help patients to receive active treatment, enhance their quality of life and improve their prognosis. Furthermore, early diagnosis of herpes simplex virus encephalitis is the key to reducing the misdiagnosis rate, mortality rate and disability rate, and combining symptoms with imaging examination findings raises the diagnostic accuracy as the disease is not clinically significant (4). The cerebrospinal fluid is produced by the choroid plexus in the ventricular system to maintain the stability of the internal environment of the nervous system. It is subject to alterations in composition and property due to pathogenic factors, providing a reference for clinical diagnosis (5). At present, there are no clinical reports on MRI changes and the expression of NSE and MCP-1 in cerebrospinal fluid of patients with severe herpes simplex virus encephalitis. Therefore, 68 patients with severe herpes simplex virus encephalitis were enrolled in this study to analyze MRI changes and the expression of NSE and MCP-1 in cerebrospinal fluid of severe patients, so as to provide a reference for early clinical diagnosis of this disease.

Materials and Methods

Research Subjects

Sixty-eight patients with severe herpes simplex virus encephalitis admitted to our hospital from April 2020 to April 2021 were enrolled as the study group, comprising 35 males and 33 females, aged 21-60 years, with a mean age of (39.27±4.08) years. Of them, 49 had HSV-I encephalitis and 19 had HSV-II encephalitis, while 43 were comatose and 25 were unconscious. Meanwhile, 68 healthy people who underwent normal physical examination in our hospital were selected as the control group, comprising 40 males and 38 females, aged 23-61 years, with a mean age of (39.87±3.62) years. The two groups were compared in terms of gender and age data ($P > 0.05$) after the families of the subjects were informed of the study and signed the consent form, and this study was approved by the ethics committee of our hospital [approval No. (2020) Ethical Review No. 23].

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Inclusion criteria: clinically diagnosed with severe herpes simplex virus encephalitis; clinically characterized by prodromal infection, headache, fever, seizures, and mental disorders; the diagnosis was confirmed by lumbar puncture, serology, electroencephalogram and cranial MRI.

Exclusion criteria: other organic lesions; expected survival <6 months; concomitant malignancy; conscious or mental disorders.

Indicator testing

Sampling

After being collected from the study group one week after the onset of disease and from the control group 2-4 days after the first lumbar anesthesia, all cerebrospinal fluid specimens were placed at room temperature for 2 h. The specimens were then centrifuged for 15 min at a radius of 15 cm and 3000 r/min to collect the supernatant serum at the end of centrifugation and store the specimens in a refrigerator at -80°C for later use.

MRI

Conventional cranial transaxial, sagittal, and coronal T1WI and T2WI scans and FLAIR sequences were performed with Philips 1.5T superconducting MRI. As for enhanced CT, transaxial, sagittal and coronal T1WI scans were performed with 0.1 mmol/kg body weight injection of Gd-DTPA contrast agent.

NSE and MCP-1 assays

ELIEA was used to detect NSE and MCP-1 expressions in the cerebrospinal fluid of two groups as follows: remove the serum specimens from the refrigerator and leave them at room temperature for 30 s, prepare the standard serum and solution, wash the plate for 30 s with 300 μL of wash solution, pat the wells dry and add 50 μL of assay buffer, standard solution, samples and detection antibodies, seal the plate, shake, and incubate the plate at room temperature (37°C) for 2 h, wash the plate, introduce 100 μL of enzyme marker into the wells, seal the plate, shake, place the plate at room temperature for 45 min, wash the plate, add 100 μL of substrate solution to the wells to incubate for 30 min in shade, add 100 μL of termination solution to shake, and measure the absorbance value at 450 nm using an enzyme marker.

Statistical treatment

SPSS 26.0 was employed to process the data, and Kolmogorov-Smirnov was used to test whether the data conformed to a normal distribution. The measurement data were described using ($\bar{x} \pm s$), and compared between groups through an independent t-test whereas the count data were expressed as %. Pearson correlation was used to analyze the correlation between NSE/MCP-1 and severe herpes simplex virus encephalitis. $P < 0.05$ was considered statistically significant.

Results

MRI

In this study, CT and MRI were performed on 68 patients with severe herpes simplex virus encephalitis. They were first performed on the second day of onset, with 9/68 cases (13.24%) presenting abnormal CT findings and 16/68 (23.53%) abnormal MRI findings within one week, and 29/68 cases (42.65%) showing abnormal CT and MRI findings within 90 days. Patients with severe herpes simplex virus encephalitis showed multiple lamellar irregular hypointense shadows in the gray matter and disappearance of the gray-white matter interface on CT, while they had long T1 and 12 lamellar signals in the gray matter with heterogeneous distribution on MRI. Most of the patients had multiple foci, all involving the lobes of the brain, and involving the temporal lobe in 9 cases (13.24%), the insula in 7 cases (10.29%), the frontal lobe in 12 cases (17.65%), the parietal lobe in 5 cases (7.35%), and the occipital lobe in 3 cases (4.41%). The limbic system was involved in 13 (19.12%), including 7 in the middle hippocampus (10.29%) and 6 in the cingulate gyrus (8.82%). Two patients had brainstem involvement (2.94%), 1 coronal involvement (1.47%), 3 basal ganglia involvement (4.41%), 5 ventricular enlargements (7.35%), 4 combined hemorrhage within the lesion (5.88%), and 2 gyrus-like and linear enhancement (2.94%). The lesions showed long T1 and long T2 signals in 21 cases (30.88%), of whom 2 (9.52%) had long T1 in 5 (23.81%) with short T1, equal T1 and long T2 in 2 cases (9.52%), and mass effect in 3 cases (14.29%). Sixty-eight patients with severe herpes simplex virus encephalitis were scanned with a fluid-attenuated inversion-recovery (FIAIR) technique, which gives better clarity of the lesion than T2WI and makes the signal of the lesion significantly different from the surrounding tissue due to identifiable and neat margins. Two cases (2.94%) presented a suspicious signal on MRI T2WI, which could not be identified as surrounding supporting tissue or lesion, but was confirmed as the lesion signal on FIAIR, as shown in Figure 1.

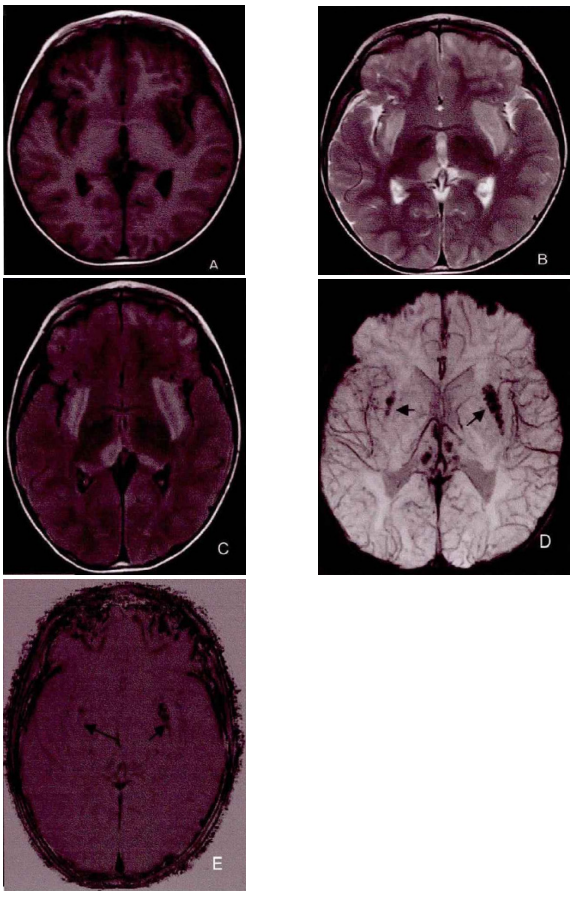

Figure 1. MRI images.

As shown in Table 1, the cerebrospinal fluid NSE and MCP-1 expressions in the study group were significantly higher than those in the control group (P < 0.05).

Correlation of NSE and MCP-1 expression with coma level in patients with severe herpes simplex virus encephalitis

As shown in Table 2, the expressions of NSE and MCP-1 were significantly higher in comatose patients with severe herpes simplex virus encephalitis than in conscious patients with severe herpes simplex virus encephalitis (P < 0.05).

Correlation between NSE and MCP-1

As shown in Figure 2, correlation analysis performed on NSE and MCP-1 showed a positive correlation between the two indicators (r=0.597, P=0.001).

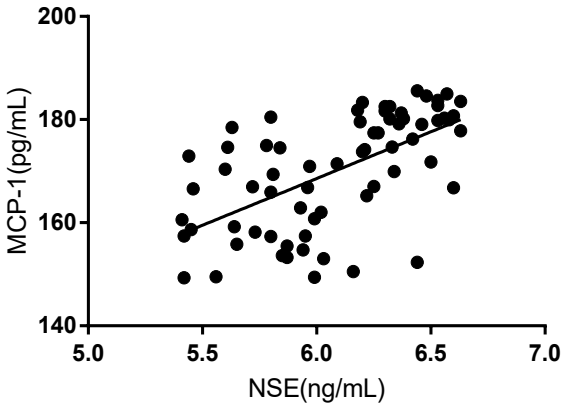

Figure 2. Correlation between NSE and MCP-1

Multiple logistic regression of factors behind the occurrence of severe herpes simplex virus encephalitis

As shown in Table 3, the severe simplex virus encephalitis was given as the dependent variable (not occurring =0, occurring =1), NSE and MCP-1 as the independent variables, the assignment increase of which was set as 1 and the normal value as 0. They were included in a dichotomous multi-factor logistic regression model. The results showed that NSE and MCP-1 were risk factors for the occurrence of severe herpes simplex virus encephalitis, indicating statistically significant differences (P < 0.05).

Discussion

Herpes simplex virus encephalitis, also known as acute necrotizing encephalitis, is caused by herpes virus infection and was characterized by such pathological changes as brain tissue edema, softening, and hemorrhagic necrosis. Its pathology determines to a certain extent the specificity of its MRI performance, so MRI helps clinical practitioners to diagnose the disease and take active treatment measures as early as possible, thereby improving the prognosis (6-7). According to previous studies(8-10), MRI showed multiple scattered lesions of variable size in patients with herpes simplex virus encephalitis and solitary lesions with multiple confined or large abnormal signals in a few cases, which may be accompanied by hemorrhage, insignificant enhancement or linear, speckled, flaky or nodular enhancement. In this study, MRI showed enhancement 2 weeks after the onset of severe herpes simplex virus encephalitis. Due to the high sensitivity of T2 spin-echo technique to increased brain water content and its advantage of multi-planar imaging without interference at the skull base, MRI was not susceptible to blur effect and skull base bone deformation to a certain extent, compared with CT. In addition, MRI showed patchy long T1 and long T2 signals in the temporal lobe, hippocampus and frontal lobe in patients with severe herpes simplex virus encephalitis, suggesting brain tissue necrosis based on the early diagnosis.

Enolase is an enzyme involved in glycolysis that consists of α, β and γ subunits. This enzyme is the main enzyme present in neurons and neural cells, which is called NSE (11-12). Previous studies (13-15) have revealed that NSE expression in cerebrospinal fluid and blood raises with the destruction of glial cells and neurons during brain damage, so changes in NSE expression are often used to predict the damage and severity of glial cells and neurons

Table 1. Comparison of cerebrospinal fluid NSE and MCP-1 expression in the two groups ($\bar{x} \pm s$).

Group	Cases	NSE (ng/mL)	MCP-1 (pg/mL)
Control	68	19.67±2.05	32.46±3.28
Study	68	6.01±0.63	165.72±16.59
t value		52.520	64.980
P value		0.001	0.001

Table 2. Correlation of NSE/MCP-1 expressions with coma levels ($\bar{x} \pm s$).

Group	Cases	NSE (ng/mL)	MCP-1 (pg/mL)
Comatose	25	9.25±0.94	107.45±10.76
Conscious	43	18.46±1.87	164.79±16.48
t value		22.950	15.550
P value		0.001	0.001

Table 3. Multiple logistic regression of factors behind the occurrence of severe herpes simplex virus encephalitis.

Indicators	*Beta*	*SE*	*Wald*	*P* value	*OR value*	95%*CI*
NSE	1.58	0.45	6.189	0.011	1.58	1.023-2.178
MCP-1	1.89	0.76	8.254	0.003	2.36	1.752-2.974

in patients with brain injury. Additionally, NSE expression is closely associated with the severity of traumatic brain injury and infarct size in patients with cerebral infarction. However, the limitation is that all of these studies were not explored in the present study, and therefore follow-up studies were needed. Here, NSE expression was significantly higher in the cerebrospinal fluid of patients with severe herpes simplex virus encephalitis, which could be regarded as an extremely sensitive indicator of brain neuron injuries. It implied that ischemic-hypoxic necrosis of brain tissue occurs in the event of an outbreak, and increased NSE expression in cerebrospinal fluid resulted in secondary cell metabolic disorders, which had a propulsive effect on neuronal injury and death.

MCP-1 is clinically recognized as one of the important chemotactic cytokines with chemotactic or activating monocytes/macrophages and plays an important role in atherosclerosis and ischemic cerebrovascular conditions. In cerebral ischemia-reperfusion injury, MCP-1 is an important functional molecular signal representing microglia in response to ischemic stimuli and may reflect excessive local inflammatory responses in brain tissue after ischemia-reperfusion (16-17). It has been reported (18-19) that MCP-1 is expressed at low levels in normal brain tissue but has increased expression in neurons and endothelial cells after cerebral ischemia, inducing the infiltration of mononuclear macrophages into the ischemic region, and participating in brain tissue injury. In this study, MCP-1 was abnormally elevated in the cerebrospinal fluid of patients with severe herpes simplex virus encephalitis, as the inflammatory immune response was induced by local brain tissue ischemia to play an important role in severe herpes simplex virus encephalitis. MCP-1 is the most important chemokine acting on monocytes/macrophages and regulating cytokine production and expression of adhesion molecules on the surface of monocytes. Hence, elevated MCP-1 expression may be associated with an excessive inflammatory response.

In conclusion, this study showed that MRI findings were significantly altered in patients with severe herpes simplex virus encephalitis, including higher NSE and MCP-1 expressions in cerebrospinal fluid, both of which were associated with coma in patients with severe herpes simplex virus encephalitis and served as risk factors for the development of severe herpes simplex virus encephalitis. However, there are still shortcomings in this study. However, there are shortcomings in this study, such as small sample size, single study population, and geographical limitation of case sources. Therefore, it is necessary to increase the sample size, include multi-center subjects and expand the source range of cases in future studies to confirm the accuracy of this study.

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