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ORIGINAL PAPER

Cerebrospinal fluid neuron specific enolase, interleukin-1β and erythropoietin concentrations in children after seizures

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Abstract
Purpose In the present study, the levels of neuron-specific enolase (NSE), interleukin-1β (IL-1β), and erythropoietin (EPO) in cerebrospinal fluid (CSF) in children with idiopathic epilepsy were measured to illuminate the relationships between these markers with idiopathic epilepsy.
Methods Eighty-five children from 6 months to 12.5 years of age with single, previously undiagnosed, and untreated idiopathic epilepsy were participated in this study. The concentrations of CSF NSE, 1L-1β, and EPO were measured by specific ELISA methods.
Results The mean concentrations of CSF NSE, IL-1β, and EPO in the epileptic groups showed a significant increase ($P < 0.01$) compared with those in the control groups. Besides, the mutual correlations of NSE, 1L-1β, and EPO were also analyzed. Results showed that there were positive correlations between the levels of IL-1β, NSE, and EPO.
Conclusions The changes of NSE, 1L-1β, and EPO level in CSF may be beneficial for the pathophysiology study of epileptic seizures and the identification and diagnosis of a seizure clinically.

Keywords Epilepsy · Cerebrospinal fluid · Neuron-specific enolase · Interleukin-1β · Erythropoietin

Introduction

Epilepsy is one of the most common chronic neurological disorders and occurs most frequently in early childhood and in older age (>60 years) [1–3]. Epilepsy is characterized by recurrent unprovoked epileptic seizures. It is a symptom of the underlying neurological disorder. The term "epilepsies" is often used to characterize multiple disorders along with epileptic seizures, so it is often difficult to figure out the exact cause of a seizure. The misdiagnosis rate in children is as high as 25% [4]. It is imperative for the experts to examine children exactly, so that accurate diagnosis may be performed early and appropriate measures could be taken in time. What is more, despite advances in pharmacological and surgical methods of treating epilepsy, its mechanisms remain poorly understood and some patients remain symptomatic despite optimal therapy currently available. A greater understanding of the pathogenesis of epilepsy would provide a fundamental basis for the development of new therapies to prevent epilepsy in the first place or modify its progress, in addition to treating its symptoms.

With the development of the researches on the pathogenesis of epilepsy, it was found that nerve-endocrine-immune network was participated in the progress of epilepsy. Researchers have found that the immune system may contribute to certain forms of epilepsy or seizure-associated disorders because of the presence of autoantibodies to voltage-gated potassium channels and glutamic acid decarboxylase [5]. And, other studies also found that the nerve and the endocrine body regulating system

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responses can take place in the brain in response to diverse insults such as seizures [6, 7]. Recently, some related biomarkers in these regulating systems were found to be useful for the diagnosis and treatment of epileptic seizures. Especially, the changes of cerebrospinal fluid (CSF) neuron-specific enolase (NSE), interleukin-1β (IL-1β), and erythropoietin (EPO) in epilepsy have caught the researchers' attention.

Enolase is a dimeric, glycolytic enzyme with three known subunit types and five isoenzymes (αα, ββ, γγ, αβ, and αγ). The distribution of these isoenzymes is well known: glial cells contain only aa-enolase, while γγ-enolase is specific for neurons (NSE). Cerebrospinal fluid neuron-specific enolase (CSF NSE) has been the best marker in adult patients with stroke as there is a strong association between high NSE and poor prognosis and has been used as a marker of neuronal damage in several CNS diseases such as stroke and anoxia, but there are only a few studies addressing CSF NSE concentrations in epilepsy. Several reports on the elevation of NSE after single seizures and status epilepticus in adults were published in the last decade, but the results were diverging [8–10]. Data in children with neurological disorders are rare.

Inflammation is an important factor in the pathophysiology of seizures and epileptogenesis, and epileptic seizures can trigger the inflammatory response. The neuronal excitability and seizure susceptibility were stimulated under inflammatory conditions, while the seizure threshold was decreased. The increasing neuronal excitability arises from an innate immune response in the brain to peripheral or CNS inflammation. Proinflammatory cytokines and their receptors are of particular interest. Interleukin-1β and its receptors are widely distributed in the brain, with the highest density in the hippocampus [11, 12], a brain region that is pivotal to epileptogenesis. The IL-1β system has been studied using the surrogate kindling model of epileptogenesis in both adult and immature rats [13]. IL-1β can enhance the degree and frequency of seizures in rats, and pharmacological blockade of IL-1β signaling leads to prevention or delay of seizures and increases the threshold for discharge induction [14, 15]. However, the change of IL-1β in children with epileptic seizures was not studied.

Erythropoietin (EPO) was first identified as a hematopoietic cytokine acting as a survival and differentiation factor [16]. Different cell types including neurons, glial cells, and endothelial cells in the nervous system produce EPO [17]. Many researches showed that EPO has a neuroprotective effect on neuronal death and apoptosis in the hippocampus of prepubertal rats after experimental model of SE. On the other hand, whether the treatment of EPO was useful for children with epileptic seizures was still unknown.

Thus, we aimed to investigate the changes of CSF NSE, 1L-1β, and EPO in children with generalized tonic-clonic seizures who had typical characteristics of epileptic seizure and electroencephalograph (EEG) and to illuminate the relationships between these markers with cryptogenic epilepsy. We hope that it can provide some values for the diagnosis and treatment for the children with cryptogenic epilepsy.

Subjects and methods

Subjects

The study was a cross-sectional, case-control study that included 85 children with generalized tonic-clonic seizures, who were divided into 3 groups according to seizure frequency and duration of a single attack. They were recruited from those following up in the Pediatric Neurology Clinic, Chair of Children and Adolescent Neurology, the Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University in the period from June 2014 to March 2015. Information on the patients' history such as age at lumbar puncture, epileptic syndrome (idiopathic/cryptogenic versus symptomatic epilepsy), magnetic resonance imaging (MRI) data, seizure semiology, duration of seizures, and time interval between seizure and lumbar puncture were extracted from the medical charts. Table 1 gives a summary of the clinical characteristics of the included patients. The diagnosis of an epileptic seizure was based on clinical history, electroencephalography, and MRI or cerebral computed tomography (CCT) data. Patients with mental retardation; abnormal findings on neurologic examination or MRI; previous history of status epilepticus; previous history of AED use; immunosuppressive therapy; autoimmune disease; thyroid, liver, or kidney disease; immune deficiency; or the presence of an infection at the time of diagnosis were excluded.

The severe group (SP) included 19 children (8 girls and 11 boys) aged between 6 and 150 months (mean 4.5 years) and all meeting the clinical criteria for SP (attacked more than 5 times within 24 h and the duration of seizures lasted more than 2 min before the first sample was collected or lasting less than 30 min one time) in a single non-prolonged seizures [18].

The moderate group (MP) included 31 children (11 girls and 20 boys) aged between 6 and 132 months (mean 4.1 years) and all meeting the clinical criteria for MP (attacked from 3 to 5 times within 24 h and the duration of seizures lasted more than 2 min before the first sample was collected or the duration ranged between 10 and 30 min) in a single non-prolonged seizures.

The light group (LP) included 35 children (17 girls and 18 boys) aged between 6 and 144 months (mean 4.2 years) and all meeting the clinical criteria for LP (attacked less than 3 times within 24 h before the first sample was collected or the duration less than 10 min) in a single non-prolonged seizures.

The control group consisted of 30 subjects (11 girls and 19 boys), aged between 6 months and 11.5 years (mean 4.2 years), selected to match the patient group for age, sex, weight, and socioeconomic aspects with no history suggesting epilepsy or other medical, neurological, or psychiatric disorders.

Table 1 Demographics characteristics of patients included in the study

Group	Age (years)[a]	Gender	Frequency of seizures	Duration of seizures
Severe group (n = 19)	4.5 (0.5–12.5)	8 female 11 male	≥5 times within 24 h	≥30 min one time
Mild group (n = 31)	4.1 (0.5–11)	11 female 20 male	3 to 5 times within 24 h	10 to 30 min one time
Light group (n = 35)	4.2 (0.5–12)	17 female 18 male	≤3 times within 24 h	≤10 min one time
Control group (n = 30)	4.2 (0.5–11.5)	11 female 19 male	No	no

[a] Data are median (range)

All patients and control subjects were informed of risks and benefits of cerebrospinal fluid examination, and they gave their written consent to participate. The study protocol was approved by the Ethics Committee of Wenzhou Medical College Hospital.

Methods

The local ethical committee approved the study, and consents were obtained from the parents of the included subjects. Detailed medical history and clinical assessment were done with special emphasis on the type of seizures, its frequency, duration of the disease, type of antiepileptic drugs, and degree of control of seizures. Patients who were seizure-free for at least 6 months were considered as "control of seizures." Other investigations needed for diagnosis and follow-up of patients with epilepsy (e.g., full blood count, liver function (aspartate aminotransferase, alanine aminotransferase, total and direct bilirubin levels) and kidney function tests, blood sugar, and electrolytes) were done for the included patients and the control groups.

NSE, EPO, and IL-1β assay

All included children were given lumbar puncture to obtain the cerebrospinal fluid specimens. After the family members gave their written consent to participate, the included patients were given the local anesthetic procaine, and the cerebrospinal fluid was taken from the clearance between the fourth and fifth lumbar intervertebral disc. Samples (1.5–2 ml) were added into the tube without heat source and endotoxin, and then centrifuged at 3000 r/min for 5 min (4 °C). The CSF samples were frozen at −80 °C until further processing.

NSE and EPO levels were measured in all samples with commercial electrochemiluminescence immunoassay and chemoluminescence, respectively. Commercially available enzyme-linked immunosorbent assay (ELISA) double antibody sandwich method was used for the measurement of CSF levels of IL-1β. Calibration was adapted to the concentrations found in CSF, with calibrations ranging from 0 to 500 pg/mL in dilute albumin-containing buffer.

Statistical analysis

Statistical calculations were carried out using statistical package for the social sciences (SPSS) 13.00. The mean and standard deviations were calculated for continuous variables. Statistical significance of differences between two groups was tested by independent two-tailed t test. Associations between continuous variables were assessed with Pearson's correlation coefficient, and a P value of <0.05 was considered significant.

Results

The present study included 85 patients with generalized tonic-clonic seizure. All the included patients were on antiepileptic drugs, either valproate (in 55 patients) or carbamazepine (30 patients), as initial monotherapy. In all, 70 patients (82.4%) did not experience seizures and were considered controlled on antiepileptic drugs. All the included patients had normal liver and kidney function results.

The mean CSF NSE level in the control group, light group, moderate group, and severe groups were 3.94 ± 1.54, 7.96 ± 2.27, 9.10 ± 2.11, and 12.50 ± 2.45 ng/ml, respectively. There is a significant increase of the level of NSE in the epilepsy groups compared with the control group (see Table 2)

The IL-1β levels in the light epilepsy group, moderate epilepsy group, severe epilepsy group (respectively, 43.53 ± 7.59, 50.80 ± 9.85, 68.42 ± 9.87 ng/ml) were significantly higher than those in the control group (8.95 ± 1.48 ng/ml) (P < 0.01). The IL-1β levels in the severe group were significantly higher than those in the moderate group and the light group (P < 0.01), and the IL-1β levels in the moderate group were higher than those in the light group (P < 0.01).

The EPO levels in the light group (3.04 ± 0.65 mU/ml) were higher than those in the control group (2.59 ± 0.74 mU/ml) (P < 0.05), and the EPO levels in the moderate and the severe group (respectively, 3.98 ± 0.72 and

Table 2 The levels of cerebrospinal fluid (CSF) neuron-specific enolase (NSE), interleukin-1β (IL-1β), and erythropoietin (EPO) in epilepsy patients

Groups	Cases	NSE (ng/ml)	IL-1β (ng/ml)	EPO (mU/ml)
Control group	30	3.94 ± 1.54	8.95 ± 1.48	2.59 ± 0.74
Light group	35	7.96 ± 2.27*	43.53 ± 7.59*	3.04 ± 0.65●
Mild group	31	9.10 ± 2.11*·***	50.80 ± 9.85*·***	3.66 ± 0.84*·***
Severe group	19	12.50 ± 2.45*·***·#	68.42 ± 9.87*·***·#	3.98 ± 0.72*·***

*$P < 0.01$, ●$P < 0.05$, compared with control group; **$P < 0.05$, ***$P < 0.01$, compared with light group; # $P < 0.01$, compared with mild group

3.66 ± 0.84 mU/ml) were much significantly higher than those in the control group ($P < 0.01$).

In addition, there was a positive correlation between CSF levels of NSE and IL-1β ($r = 0.681$, $P < 0.01$) (see Fig. 1), and there was a positive correlation between CSF NSE and EPO levels ($r = 0.452$, $P < 0.01$) (see Fig. 2). What is more, a positive correlation between CSF IL-1β and EPO levels ($r = 0.407$, $P < 0.01$) was also observed in the study (see Fig. 3).

Discussion

Seizures are common in infancy and early childhood and usually have a good prognosis. The hypothetical influence of these seizures on the neuronal metabolism has been investigated previously by several authors, whose results are comparable with those obtained in the present study. Rabinowicz et al. [19] found that CSF obtained within 24 h of epilepticus showed increasing concentrations of NSE in 15 patients, which was consistent with our result. In addition, we measured CSF IL-1β and EPO levels and found significant difference of IL-1β and EPO levels between the seizures groups and control groups. These results suggested that CSF NSE, IL-1β, and EPO levels were significantly changed during epileptic seizures, which provided some hints that the nerve-immune-endocrine systems were involved in the pathological process of epileptic seizures.

Theoretically, NSE is an ideal indicator of neuronal and glial damage, since it is present only in low concentrations outside the nervous system. CSF NSE has been the best indicator of neuronal damage in such disorders as anoxic brain damage after cardiac arrest, stroke, and some specific cases of coma [20]. It has been suggested that seizure activity in the brain causes a release of NSE into the CSF compartment [21]. In our study, the CSF NSE levels were significantly increased after epileptic seizures compared with those in the control group, which indicated that epilepsy could impair the function and metabolism of neurons and cause neuronal damage, and even light epilepsy could lead to the neuronal injury. Furthermore, there were significant correlations between the seizure degree and cerebrospinal fluid neuron-specific enolase levels when analyzing all of the seizure patients. And, the mean CSF NSE levels of moderate and severe groups showed a tendency to be higher than the light group. This result was consistent with the research by Hong et al. [22], who reported that the level of CSF NSE was associated with the severity of seizures. So, the incidence of increase in concentrations of CSF NSE levels in different epileptic groups was a good indicator of neuronal and glial damage in children with seizures.

Clinical and experimental findings [23–25] support the idea that inflammatory reaction is a major pathological basis for epileptic seizure. The brain inflammation contributes to the generation and progression of epilepsy, which, in turn, activates further inflammation, thereby establishing a vicious cycle of events that contributes to the development of epilepsy. Inflammation in the brain is characterized by infiltration of circulating immune cells such as neutrophils and monocytes, and by activation of resident cells, including microglial cells, astrocytes, and endothelial cells. These cells can express, release, and respond to typical pro-inflammatory mediators like cytokines [5]. Abundant experimental researches have revealed that cytokines can modulate neuronal activity and viability by activating the neuronal receptors in the brain and spinal cord [26, 27] or by promoting the release of neuroactive molecules from glia or the endothelium (e.g., nitric oxide, glutamate, prostaglandins) [28, 29]. In the nervous system, one of the most widely studied cytokine mediators is the

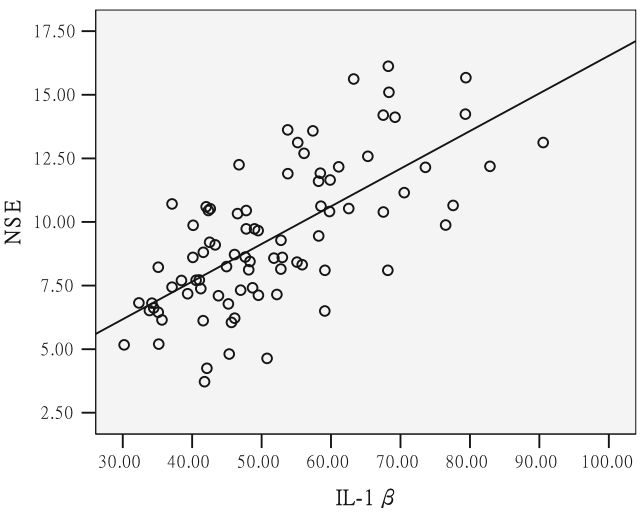

Fig. 1 Related scatter plot between CSF NSE level and IL-1β level ($r = 0.681$, $P < 0.01$, $n = 85$)

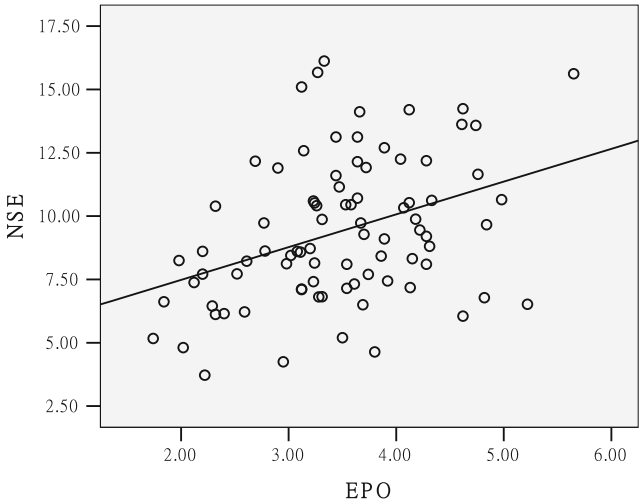

Fig. 2 Related scatter plot between CSF NSE level and EPO level ($r = 0.465$, $P < 0.01$, $n = 85$)

pro-inflammatory cytokine interleukin-1β (IL-1β), and there is considerable evidence indicating that it is a key contributor to a diverse range of neurodegenerative conditions. A series of researches have showed that the expression of IL-1β is dramatically increased in many neurodegenerative diseases such as hypoxic/ischemic, Alzheimer's disease, Parkinson's disease, stroke, and subarachnoid hemorrhage (SAH) [30–32]. IL-1β has pleiotropic effects. It exerts direct effects on neurotransmission; glia and endothelial cells stimulate the synthesis of some growth factors, neuropeptides, and some anti-inflammatory and anticoagulant cytokines such as TGF-β, IL-10, and IL-4 and also induce other pro-inflammatory cytokines like TNF-α, IL-6, IL-8, IL-2, or IFN-g [33]. Recent studies have shown high levels of IL-1β expression in the hippocampi of epileptic patients and in a rat model and are thought to play a vital role as a key regulator of immune and inflammatory response in the development of seizures and

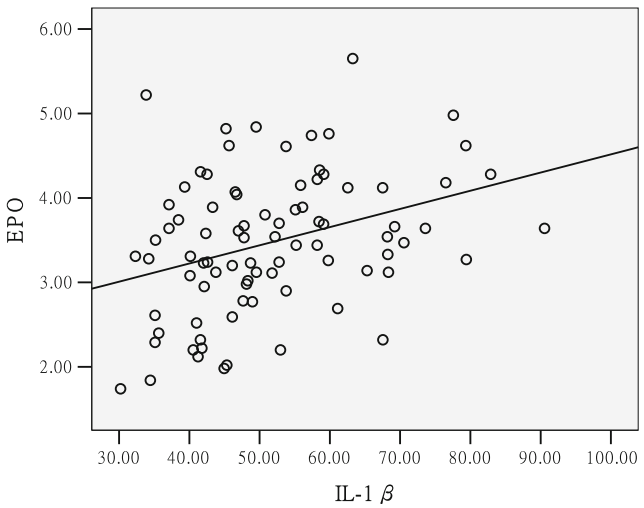

Fig. 3 Related scatter plot between CSF IL-1β level and EPO level ($r = 0.405$, $P < 0.01$, $n = 85$)

epilepsy [34, 35]. In our study, we gave an investigation of the change of CSF IL-1β level in the children after seizure, and the result showed that the level of CSF IL-1β in the children after seizure was significantly increased compared with that in the control group. In addition, according to our study, there was a positive correlation between IL-1β and the NSE ($r = 0.681$, $P < 0.01$) (Fig. 1), which may explain why CSF 1L-1β levels show a significant positive correlation with the duration and frequency of seizures. IL-1β as an epileptogenic factor not only associated with the presence of epileptic seizures but also positively correlated with the severity of epilepsy. However, the clinical data of further dynamic observation of 1L-1β level with epilepsy seizure after 24 h later were not provided.

Erythropoietin (EPO) was originally identified as a kidney-derived stimulator of erythroid progenitor cell proliferation and differentiation [36]. However, several lines of evidences suggested that EPO and erythropoietin receptor (EPOR) were expressed by other tissues, including the nervous system. Different cell types including neurons, glial cells, and endothelial cells in the nervous system produced EPO and expressed EPOR [37]. It was an important pathogenic component, induced either by the production of cytokines and chemokines followed by leukocyte infiltration or by glial activation and proliferation [38]. Several studies demonstrated that EPO could enhance phagocytosis in polymorphonuclear cells and reduce the activation of macrophages, thus modulating the inflammatory process. Juul et al., for example, demonstrated that EPO concentration in the CSF was significantly higher in newborns with asphyxia or intraventricular hemorrhage than in controls, and this increase was related to an enhanced CNS synthesis of EPO rather than any passage through the BBB [39]. Nichol et al. observed that EPO was increased in the CSF of five patients with traumatic brain injuries [40]. Also, the concentrations of CSF-EPO in patients with depression were higher than those in controls. One suggested hypothesis was that the brain of a patient with depression may be in a state of hypoxia, which may induce EPO production [41]. Besides, EPO also ameliorated the latency and severity of seizures and significantly increased survival in the kainate-induced experimental seizure model. Thus, EPO may provide benefit in epileptic disorders [42]. In our study, we found that CSF EPO levels in epilepsy seizure were much higher than those in the control group, which suggested that EPO could play a protective role in neuronal survival after an epileptic seizure. EPO levels in the moderate and severe group were significantly higher than those in the light group ($P < 0.01$) and showed a positive correlation with NSE ($r = 0.452$, $P < 0.01$) (Fig. 2), but there was no statistical significant difference in the EPO levels between the severe group and the moderate group ($P > 0.05$). The results may be relevant to that under anaerobic conditions, the severe ischemic brain tissue secreted less than enough EPO, or the time

needed for new synthetic EPO was too long and EPO cell function was impaired. All these may lead to the EPO synthesis and secretion disorder after severe hypoxia and ischemia of brain tissue thus to prevent the EPO levels continued to rise. Most important, there was a positive correlation between 1L-1β and EPO ($r = 0.407$, $P < 0.01$) (Fig. 3). Krajewski et al. reported that IL-1β, at least partially, reduces hypoxia-induced EPO expression by downregulation of HNF-4a [43]. On the other hand, Thornton et al. found that EPO could attenuate brain injury and attenuate the high expression of IL-β mRNA induced by hypoxia/ischemia [44]. A tight connection was observed between IL-1β and EPO, which may play an important role in the brain injury-protection mechanism. IL-1β may serve as a promoting inflammatory immune cell factor, while EPO was considered as a paracrine and autocrine endocrine factor, which indicated that the nerve-endocrine-immune system may play a key role in epilepsy, but the specific mechanism still needs more researches to prove it.

In conclusion, we reported that CSF NSE, IL-1β, and EPO levels changed after epilepsy seizure compared with those in the control group. In the pathogenesis of epilepsy, CSF NSE as an indicator of neuronal and glial damage, IL-1β as promoting inflammatory immune cell factors, and EPO as paracrine and autocrine endocrine factors, which indicated that the nerve-endocrine-immune system, may play a key role in children with epilepsy seizure. But, more researches are needed to prove their specific mechanism.

Compliance with ethical standards

Conflict of interest There are no conflicting interests of this paper.

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