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Original Article

The increase of serum NSE and TBil levels related to the severity of HIBD

Weiyan Zhang, Caiyu Hu, Juhua Ji, Fei Hong, Meijun Zhu, Cheng Xu, Xiaohong Yang, Xiaojuan Sun, Lei Ye, Lei Song

Department of Pediatrics, The Second Affiliated Hospital of Nantong University, Nantong First People's Hospital, Nantong, Jiangsu, China

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Abstract: Objective: To investigate the significance of amplitude integrated electroencephalogram (aEEG), neuron-specific enolase (NSE) and total bilirubin (TBil) in the diagnosis and treatment of neonatal hypoxic-ischemic brain damage (HIBD). Method: Ninety infants with HIBD in our hospital were selected as the study subjects (group A), and 90 patients with normal full-term delivered in our hospital were selected as control group B. Group A was given hyperbaric oxygen therapy. The aEEG background activities of group A before and after treatment and group B were examined. The levels of NSE and TBil in the serum of group A before and after treatment and also in group B were detected by automatic biochemical analyzer. Spearman correlation coefficient was used to analyze the correlation between serum NSE and TBil levels and aEEG background activity. Results: The abnormal rate of background activity of group A aEEG was higher in Group A than that of group B ($P<0.001$). The levels of serum NSE and TBil in group A were higher than those in group B ($P<0.001$). The levels of serum NSE and TBil were positively correlated with background activity of aEEG ($r=0.514$, $P<0.001$; $r=0.455$, $P<0.001$). The effective rate of treatment for infants with normal aEEG background activity and mild abnormalities in group A was higher than that in infants with severe aEEG background activity ($P<0.001$). The treatment ineffectiveness of infants with a background activity of aEEG and mild hypoplasia was lower than that of infants with severe aEEG background activity ($P<0.001$). After treatment the serum levels of NSE and TBil were lower in group A than before treatment ($P<0.001$). Conclusion: The increase of serum NSE and TBil levels may be closely related to the severity of HIBD. Observation of aEEG background activity, serum NSE and TBil levels is of certain guiding significance for the treatment of HIBD in infants with hyperbaric oxygen.

Keywords: Neonatal brain injury, aEEG, neuron specific enolase, bilirubin

Introduction

HIBD is a common tissue-damaging disease at birth, affecting about 1-2% of full-term live births [1]. The occurrence of HIBD includes congenital or metabolic diseases, hypoxie-ischemic encephalopathy (HIE), asphyxia, and the like. Patients with severe disease may have neurological functional disorders such as cognitive impairment, developmental delay, and cerebral palsy, which are important factors in causing neurobehavioral damage and death in neonates [2]. With the development of intensive care technology, the survival rate of high-risk newborns increases year by year, but the incidence of neurobehavioral damage has not decreased [3]. Therefore, early detection of abnormal brain function and degree of brain injury in high-risk neonates and timely and effective brain protection therapy are of great significance for improving the quality of life of neonates.

At present, comprehensive evaluation of neonatal HIBD brain injury is mainly based on clinical symptoms, EEG, cephalic ultrasound, Apgar score, etc. [4]. However, these evaluation methods are subjective and lack specificity, and cannot detect neonatal brain dysfunction in time, thus missing best treatment opportunity [5]. aEEG is a clinically simple and effective way to evaluate neurological function, and is an auxiliary monitoring method for brain function and severity of brain injury in critically ill patients [6].

NSE, a key enzyme in the cytoplasm of nerve cells, cannot be secreted by neurons to the

extracellular spsce under normal physiological conditions. In the case of hypoxia and ischemic brain injury, it can enter the peripheral blood through the blood-brain barrier due to the increased permeability of the blood-brain barrier [7]. Bilirubin's brain injury mechanism is relatively complex, involving multiple levels and multiple factors, which can not only cause nuclear jaundice, but also cause brain injury [8].

In recent years, studies have confirmed that hyperbaric oxygen therapy has significant clinical effects on brain injury [9]. Hyperbaric oxygen can increase blood oxygen concentration, and can prevent the brain from being affected by hypoxia and ischemia when brain injury occurs in newborns. It can promote the recovery of brain damage and repair the damaged brain cells of the child [10]. There have been many studies on brain damage in aEEG, NSE and bilirubin [11-13], but there are few studies on the relationship between aEEG background activity and NSE and bilirubin, and their role in hyperbaric oxygen therapy in neonatal HIBD. In this study, aEEG, serum NSE and TBil were detected in infants with HIBD to explore the roles of these three factors in the early onset and treatment of infants with HIBD.

Materials and methods

General information

Nintey infants with HIBD in our hospital were selected as subjects (group A), including 55 males and 35 females. The gestational age was 38-41 weeks, the average gestational age was (39.6±2.1) weeks, the birth weight was 2500-4400 g, and the average body mass was (2953.9±123.4) g. In addition, 90 patients with normal full-term newborns who were delivered in our hospital during the same period were selected as control group B. There were 47 males and 43 females with a gestational age of 37-41 weeks, an average gestational age of (39.1±2.5) weeks, a birth weight of 2540-4360 g, and an average body weight of (2967.6± 116.9) g. This study was approved by the ethics committee of the Second Affiliated Hospital of Nantong University, Nantong First People's Hospital. The parents of the infants were informed of the study and they signed informed consent forms.

Inclusion and exclusion criteria

Inclusion criteria: infants born in our hospital; group A met the diagnostic criteria of HIBD in the Chinese Medical Association's Diagnostic Criteria for Neonatal Hypoxic-ischemic Encephalopathy [14], and confirmed as HIBD by imaging studies such as MRI, CT and ultrasound. Group A had an Apgar score of ≤3 at 1 min after birth and an Apgar score of ≤5 at 5 min after birth; Exclusion criteria: patients with congenital neurological disease, patients with circulatory system disease, patients with glycemic encephalopathy, patients with central nervous system infection, patients with genetic metabolic disease, patients with respiratory disease, patients with congenital neurological disease, patients with intrauterine TORCH infection, patients with glycemic encephalopathy, patients with congenital malformation, hypocalcemia or malignant tumor; patients with a history of familial deafness.

Treatment and evaluation

Group A was given symptomatic treatment, including oxygen, anti-infection, warmth, electrolytes, acid-base balance, and Vojta-induced maintenance of water. They were treated in an YLC0.5/1.2 infant oxygen chamber (Wuhan Ship Design and Research Institute Co., Ltd., China). The cabin pressure was 0.14-0.2 MPa9, which was pressurized for 20 min, stabilized for 30 min, and decompressed for 20 min, once a day. The treatment was 5 days a week for a single course of treatment, and 6 courses for a continuous treatment.

The clinical efficacy of the treatment was assessed using the Gesell Developmental Schedules (Gesell) [15]. The DQ values after 12 weeks were evaluated, wherein a DQ value of >85 was normal, a DQ value of 76-85 was in a marginal state, and a DQ value of <76 was a developmental delay. A DQ value of >85 points was considered as markedly effective, a DQ value of 76-85 points was effective, a the DQ value <76 points was ineffective. (cases of markedly effective + cases of effective)/total cases 100% = effective.

aEEG monitoring

The infants were monitored with a Nicolet One Brain Function Monitoring (Nanjing Yihe

Industrial Co., Ltd., China). According to the 10-20 international standard [16], electric shock was administered on the scalp, aligned the top area (C-C4), the central area CZ, the occipital area (O1-O2), the forehead area (FP1-FP2) and the sacral area (T3-T4) as a collection point. The electrode placement sites are cleaned with a degreaser to reduce electrical resistance. The electrode cap was fixed with an electrode paste. The brain function monitor is turned on to output with a resistance of less than 20 ohms and 6 cm/h. The amplitude band of the spectrum was scanned for 12-24 h each time. When the lower boundary of the amplitude waveform was >5 μV and the upper boundary was >10 μV, the amplitude was normal; when the lower boundary of the amplitude waveform was <5 μV and the upper boundary was >10 μV, it was a mild anomaly; when the lower boundary of the amplitude waveform was <5 μV and the upper boundary was <10 μV, it was a severe anomaly.

Serum NSE and TBil detection

In the morning, 3 mL of venous blood was extracted from the subjects on an empty stomach and placed in vacuum blood collection without anticoagulant. Serum was separated by centrifugation, and NSE (chemical method) and TBil (vanadate oxidation method) in serum were detected by Hitachi 7600-020 automatic biochemical analyzer. The kit was purchased from Shanghai Yaji Biotechnology Co., Ltd., China. The testing process was carried out in strict accordance with the instructions of the instrument and kit.

Statistical method

SPSS 22.0 was used for statistical analysis (IBM Corp, Armonk, NY, USA). The count data was expressed in terms of number/percentage [n (%)]. The chi-square test was used to compare the count data between groups. When the theoretical frequency in the chi-square test was less than 5, the continuous calibration chi-square test was used. Measurement data were expressed as mean ± standard deviation ($\bar{x} \pm sd$). The independent sample t test was used to compare the measurement data between groups, and the paired t test was used for comparison before and after the group. Spearman correlation coefficient was used to analyze the correlation between serum NSE and TBil levels and aEEG background activity. When $P<0.05$, the difference was considered statistically significant.

Result

General information of both groups

There was no significant difference in general clinical data between group A and group B ($P>0.05$), including gender, gestational age, birth weight, mode of delivery, head width, length, first feeding time, parity, premature rupture of membranes, placental abruption, gestational diabetes mellitus, gestational hypertension, maternal residence, scaring of the uterus, and irregular labor test (**Table 1**).

Abnormal rates of aEEG background activity in both groups

In group A, there were 22 cases with normal aEEG background activity (24.44%), 57 cases with mild abnormality (63.34%), and 11 cases with severe abnormality (12.22%), with an abnormality rate of 75.56%. In group B, there were 90 cases normal aEEG background activity (100.00%), without any abnormality. The abnormal rate of aEEG background activity in group A was significantly higher than that in group B ($P<0.001$) (**Table 2**).

Serum NSE and TBil levels in both groups

Serum NSE levels in group A were significantly higher than those in group B ($P<0.001$); serum TBil levels in group A significantly were higher than those in group B ($P<0.001$) (**Table 3**; **Figure 1**).

Correlation between serum NSE and TBil levels and background activity of aEEG in group A

In group A, normal aEEG background activity was set as 1, mild abnormality was set as 2, and severe abnormality was set as 3. Spearman correlation analysis was conducted between serum NSE and TBil levels and aEEG background activity. Serum NSE level was positively correlated with aEEG background activity ($r=0.514$, $P<0.001$). Serum TBil level was positively correlated with aEEG background activity ($r=0.455$, $P<0.001$) (**Figure 2**).

Relationship between aEEG background activity and therapeutic outcome

After treatment, 81 cases (90.00%) in group A showed a score of markedly effective, 4 cases

Table 1. General information of Group A and Group B [n (%)]/($\bar{x}$ ±sd)

Category	Group A (n=90)	Group B (n=90)	t/χ^2 value	*P* value
Gender			1.448	0.229
Male	55 (61.11)	47 (52.22)		
Female	35 (38.89)	43 (47.78)		
Gestational age (week)	39.6±2.1	39.1±2.5	1.453	0.148
Birth weight (g)	2953.9±123.4	2967.6±116.9	0.765	0.446
Mode of delivery			0.217	0.642
Production	56 (62.22)	59 (65.56)		
Cesarean section	34 (37.78)	31 (34.44)		
Head circumference (cm)	33.5±3.6	34.1±3.8	1.087	0.278
Length (cm)	48.6±2.8	48.9±2.4	0.772	0.441
First feeding time (h)			0.833	0.361
<24	33 (36.67)	39 (43.33)		
≥24	57 (63.33)	51 (56.67)		
Fetal times			0.564	0.453
First child	48 (53.33)	53 (58.89)		
Second child and above	42 (46.67)	37 (41.11)		
Premature rupture of membranes			0.850	0.356
Yes	21 (23.33)	16 (17.78)		
No	69 (76.67)	74 (82.22)		
Placental abruption			0.719	0.396
Yes	15 (16.67)	11 (12.22)		
No	75 (83.33)	79 (87.78)		
Pregnancy gestational diabetes			0.585	0.444
Yes	10 (11.11)	7 (7.78)		
No	80 (88.89)	83 (92.22)		
Pregnancy gestational hypertension			0.310	0.578
Yes	8 (8.89)	6 (6.67)		
No	82 (91.11)	84 (93.33)		
Pregnant mother's place of residence			0.259	0.611
City	65 (72.22)	68 (75.56)		
Rural	25 (27.78)	22 (24.44)		
Scar uterus			0.392	0.531
Yes	15 (16.67)	12 (13.33)		
No	75 (83.33)	78 (86.67)		
Irregular birth check			1.338	0.247
Yes	5 (5.56)	2 (2.22)		
No	85 (94.44)	88 (97.78)		

(4.44%) were effective, 5 cases (5.56%) were ineffective, and the effective rate was 94.44%. In group A, the treatment efficiency of infants with normal aEEG background activity and mild abnormal aEEG background activity was higher than that of infants with severe aEEG background activity (P<0.001). Infants with normal aEEG background activity and mild abnormal aEEG background activity in group A had less effective treatment than those with severe aEEG background activity (P<0.001) (**Table 4**).

Serum NSE and TBil levels before and after treatment in group A

After treatment, serum NSE level was significantly lower than that before treatment (P<0.001) in group A. Serum TBil level was sig-

Table 2. Comparison of abnormal rate results of aEEG background activities between Group A and Group B [n (%)]

Group	n	Normal	Mild abnormality	Severe abnormality	Abnormal rate (%)
Group A	90	22 (24.44)	57 (63.34)	11 (12.22)	75.56
Group B	90	90 (100.00)	0 (0.00)	0 (0.00)	0.00
χ^2 value	-	109.310	80.511	9.683	16.110
P value	-	<0.001	<0.001	0.002	<0.001

Table 3. Comparison of serum NSE and TBil levels in group A and group B ($\bar{x}$ ±sd)

Group	N	NSE (ng/mL)	Tbil (μmol/L)
Group A	90	32.86±8.95	59.68±10.42
Group B	90	9.10±3.05	38.75±8.26
t value	-	23.840	14.930
P value	-	<0.001	<0.001

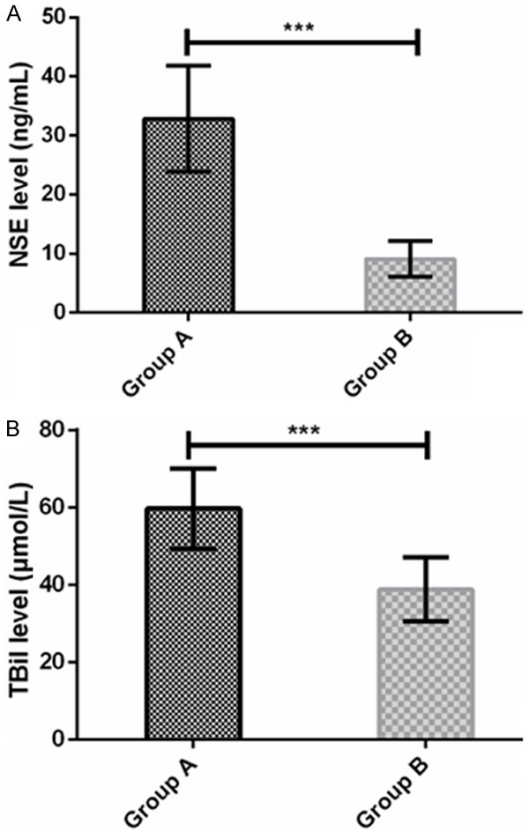

Figure 1. Comparison of serum NSE and TBil levels in groups A and B. Comparison of serum NSE levels between group A and group B (A); The results of serum TBil levels in group A and group B were compared (B). Note: ***P<0.001.

nificantly lower after treatment than that before treatment (P<0.001) (**Figure 3**).

Discussion

With the development of intensive care technology and the clinical application of pulmonary surfactant and corticosteroids in newborns, the survival rate of high-risk full-term newborns has been greatly improved, but the incidence of neonatal HIBD has not decreased [2]. HIBD can cause neurological disability in neonates, and some have no typical clinical symptoms at the onset of the disease. If there is no symptomatic treatment, related complications and sequelae in the subsequent growth and development can seriously affect the growth of newborn babies and their families [17]. Therefore, early monitoring of neonatal HIBD and timely and effective intervention therapy are of great significance for improving the disease status of neonatal HIBD.

Clinical imaging such as CT and MRI can accurately determine the location, type and extent of intracranial lesions. It has non-invasive, multi-planar and multi-parametric imaging features and is a well-recognized intracranial lesion examination [18]. However, the application in the early stage of HIBD has certain limitations, the device cannot move and lacks certain specificity for early HIBD [19].

aEEG is a simplified EEG monitoring system. It can collect EEG signals at the biparietal bone, and the collected data is output on heat-sensitive paper through amplitude compression, filtered and integrated by frequency, and output is on heat-sensitive paper [20]. aEEG has the advantages of simple operation and small external interference. The results can be used to analyze the condition of brain injury and have great value in brain function monitoring of HIBD [21]. NSE is a glycolytic enzyme, its composition is made of acidic protein, which is a strong marker of neuron damage, and its level can be used to judge the degree and prognosis of brain injury [22]. Hypoxic asphyxia and acidosis in the body affect liver enzyme activity, which reduces the binding force of albumin and bilirubin. In turn, the bilirubin metabolic capacity is impaired, resulting in the elimination of bilirubin, which can cause an increase in bilirubin in the body [23]. There have been many studies on brain damage in aEEG, NSE and bilirubin. As in Soubasi et al. [24], the early aEEG characteris-

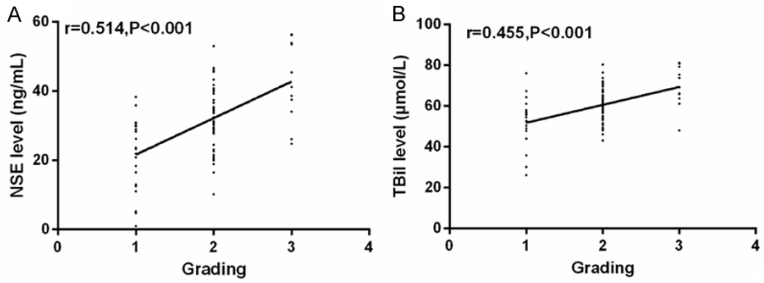

Figure 2. Correlation between serum NSE and TBil levels in group A and background activity of aEEG. Correlation between serum NSE levels in group A and background activity of aEEG (A); Correlation between serum TBil levels in group A and background activity of aEEG (B).

Table 4. Relationship between background activity and therapeutic effect of aEEG in group A [n (%)]

Category	n	Significant effect	Effective	Invalid
Normal	22	22 (100.00)	0 (0.00)	0 (0.00)
Mild abnormality	57	54 (94.74)	2 (3.51)	1 (0.00)
Severe abnormality	11	5 (45.45)***	2 (18.18)	4 (36.36)***
χ^2 value	-	28.122	5.947	22.762
P value	-	<0.001	0.051	<0.001

Note: ***P<0.001 compared with normal, and mild abnormalities.

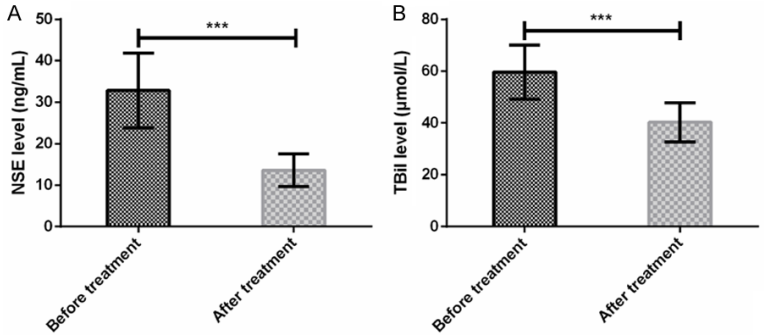

Figure 3. Comparison of serum NSE and TBil levels before and after treatment in group A. Comparison of serum NSE levels before and after treatment in group A (A); The results of serum TBil levels before and after treatment in group A were compared (B). Note: ***P<0.001.

tics of premature infants with severe head injury can be used as a prognostic indicator for preterm infants with severe brain injury. In the study by Çeltik et al. [25], NSE is a marker reflecting the severity of HIE. Serum NSE levels were significantly elevated in HIE infants, which were associated with poor short-term prognosis. In Luo et al. [26], serum bilirubin levels after acute ischemic stroke were associated with stroke severity. Thus, aEEG, NSE and bilirubin have important detection value in brain injury. In this study, the abnormal rate of background activity of group A aEEG was significantly higher than that of group B. The levels of serum NSE and TBil in group A were significantly higher than those in group B, and serum NSE and TBil levels were positively correlated with background activity of aEEG. The severity of the condition of infants with HIBD can be judged according to the abnormal rate of background activity of aEEG. The increase in NSE and TBil levels is closely related to the severity of the disease in infants with HIBD. In the study Zhang et al. [27], dynamic monitoring of TNF-α, HMGB1, NSE values, combined with aEEG, can provide a useful basis for early diagnosis, severity judgment and short-term prognosis of neonatal asphyxia brain injury, which is similar to our study. Therefore, observing aEEG background activity and serum NSE and TBil levels are important for the monitoring of early neonatal HIBD.

At present, neonatal brain injury is mostly treated by asphyxiation ABCDE resuscitation, but the sequela incidence and fatality rate of neonatal brain injury are still high [28]. Hyperbaric oxygen therapy is able to improve oxygen partial pressure, increase blood oxygen content and blood oxygen diffusion time and range, as well as improve oxygen metabolism, which promotes the establishment of collateral circulation. This, in turn, improves the oxygen supply to brain tissue and can prevent cerebral edema and brain injury caused by reperfusion [29]. Much research has been done on the neonatal brain injury of hyperbaric oxygen. Liu et al. [30] concluded from systematic evaluation that hyperbaric oxygen therapy can reduce the mortality and neurological sequela of HIE full term newborns. Also, Zhou et al. [31] found that hyperbaric oxygen therapy for HIE was safe and

effective, and can reduce the mortality and disability caused by HIE. As the pressure increased, the infants' antioxidant capacity was significantly enhanced. In this study, the treatment efficiency of infants with HIBD after treatment with hyperbaric oxygen reached 94.44%. This confirmed that hyperbaric oxygen therapy was an effective treatment for infants with HIBD. The effective rate of treatment for infants with normal aEEG background activity and mild abnormalities in group A was significantly higher than that in infants with severe aEEG background activity. However, their treatment inefficiency was significantly lower than that of infants with severe aEEG background activity, and serum NSE and TBil levels were significantly lower than before treatment. This suggests that aEEG background activity and NSE and TBil levels have a certain evaluation value for the treatment of infants with HIBD. In the study by Azzopardi et al. [32], aEEG is one of the reliable indicators for predicting neuronal outcome after full term newborns with HIE and has an appreciative value in neonatal hypothermia therapy.

In the study of Sun et al. [33], mild hypothermia can reduce the levels of NSE and S-100 protein in cerebrospinal fluid of HIE infants, while NSE and s-100 are significantly increased in CSF of infants with severe neurological dysfunction (with an intelligence development index or physical development index <70). It can be seen that observing aEEG background activity and NSE and TBil levels may have certain guiding value for hyperbaric oxygen therapy in infants with HIBD. However, hypothermia therapy and hyperbaric oxygen therapy are two different treatments. Mild hypothermia is a physical way to cool infants. But both are neuroprotective to neonatal brain injury [34-36].

This study confirmed the role of aEEG, serum NSE and TBil in the early assessment and treatment of infants with HIBD, but there were still deficiencies in the experiment. First, changes in aEEG, NSE, and TBil at different time points were not observed in infants with HIBD. Second, no control group was set for hyperbaric oxygen therapy, and a corresponding randomized controlled experiment was performed. These shortcomings need to be further supplemented in future research, to further support the results of this study.

In summary, the severity of neonatal HIBD can be assesed according to the abnormal rate of background activity of aEEG, and the increase of serum NSE and TBil levels may be closely related to the severity of HIBD. Observation of aEEG background activity, serum NSE and TBil levels is of certain guiding significance for the treatment of HIBD infants with hyperbaric oxygen.

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Disclosure of conflict of interest

None.

Address correspondence to: Lei Song, Department of Pediatrics, The Second Affiliated Hospital of Nantong University, Nantong First People's Hospital, No. 6 Haier Lane North Road, Chongchuan District, Nantong, Jiangsu, China. Tel: +86-15162866661; E-mail: song423666@163.com

References

[1] Xu LX, Tang XJ, Yang YY, Li M, Jin MF, Miao P, Ding X, Wang Y, Li YH, Sun B and Feng X. Neuroprotective effects of autophagy inhibition on hippocampal glutamate receptor subunits after hypoxia-ischemia-induced brain damage in newborn rats. Neural Regen Res 2017; 12: 417-424.

[2] Lai Z, Zhang L, Su J, Cai D and Xu Q. Sevoflurane postconditioning improves long-term learning and memory of neonatal hypoxia-ischemia brain damage rats via the PI3K/Akt-mPTP pathway. Brain Res 2016; 1630: 25-37.

[3] Hagberg H, David Edwards A and Groenendaal F. Perinatal brain damage: the term infant. Neurobiol Dis 2016; 92: 102-112.

[4] Varghese B, Xavier R, Manoj VC, Aneesh MK, Priya PS, Kumar A and Sreenivasan VK. Magnetic resonance imaging spectrum of perinatal hypoxic-ischemic brain injury. Indian J Radiol Imaging 2016; 26: 316-327.

[5] Ferriero DM. Neonatal brain injury. N Engl J Med 2004; 351: 1985-1995.

[6] Río RD, Ochoa C, Alarcon A, Arnáez J, Blanco D and Garcíaalix A. Amplitude integrated electroencephalogram as a prognostic tool in neonates with hypoxic-ischemic encephalopathy:

a systematic review. PLoS One 2016; 11: e0165744.
[7] Kelen D, Andorka C, Szabo M, Alafuzoff A, Kaila K and Summanen M. Serum copeptin and neuron specific enolase are markers of neonatal distress and long-term neurodevelopmental outcome. PLoS One 2017; 12: e0184593.
[8] Maduemem K, Khalid S, Hariharan M and Siddique A. Intraventricular haemorrhage complicated by hydrocephalus in an acutely encephalopathic preterm infant. Cureus 2018; 10: e2193.
[9] Hu Q, Manaenko A, Xu T, Guo Z, Tang J and Zhang JH. Hyperbaric oxygen therapy for traumatic brain injury: bench-to-bedside. Med Gas Res 2016; 6: 102-110.
[10] Jain KK. Physical, physiological, and biochemical aspects of hyperbaric oxygenation. 2017; 11-22.
[11] Zhu X, Guo Y, Liu Y and Liu K. Amplitude-integrated electroencephalography for early diagnosis and prognostic prediction of hypoxic encephalopathy in preterm infants. Med Sci Monit 2018; 24: 8795-8802.
[12] Wang Q, Wang G, Lu X, Guo X, Du Q, Lin F, Fan Q, Zhang G and Wang D. A correction formula for neuron-specific enolase measurement in hemolyzed neonatal serum samples. Biomed Rep 2018; 8: 491-496.
[13] Binnun A, Mimouni FB, Kasirer Y, Schors I, Schimmel MS, Kaplan M and Hammerman C. Might bilirubin serve as a natural antioxidant in response to neonatal encephalopathy? Am J Perinatol 2018; 35: 1107-1112.
[14] Group of Neonatology; Chinese Pediatric Society; Chinese Medical Association. Diagnostic criteria for neonatal hypoxic-ischemic encephalopathy. Zhonghua Er Ke Za Zhi 2005; 43: 584.
[15] Lv HY, Wu SJ, Wang QL, Yang LH, Ren PS, Qiao BJ, Wang ZY, Li JH, Gu XL and Li LX. Effect of erythropoietin combined with hypothermia on serum tau protein levels and neurodevelopmental outcome in neonates with hypoxic-ischemic encephalopathy. Neural Regen Res 2017; 12: 1655-1663.
[16] Goenka A, Boro A and Yozawitz E. Comparative sensitivity of quantitative EEG (QEEG) spectrograms for detecting seizure subtypes. Seizure 2018; 55: 70-75.
[17] Tang B, Wang D, Wu Q, Li M, Yang Q and Chen C. Role of extracellular signal-related protein kinase 1/2 pathway in ginsenoside rg1 mediated anti-apoptotic effect on neuron after hypoxia ischemia brain damage in neonatal rats. Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi 2016; 30: 1011-1018.
[18] Placha K, Luptakova D, Baciak L, Ujhazy E and Juranek I. Neonatal brain injury as a consequence of insufficient cerebral oxygenation. Neuro Endocrinol Lett 2016; 37: 79-96.
[19] Bano S, Chaudhary V and Garga UC. Neonatal Hypoxic-ischemic encephalopathy: a radiological review. J Pediatr Neurosci 2017; 12: 1-6.
[20] Hellström-Westas L. Amplitude-integrated electroencephalography for seizure detection in newborn infants. Semin Fetal Neonatal Med 2018; 23: 175-182.
[21] Jan S, Northington FJ, Parkinson CM and Stafstrom CE. EEG monitoring technique influences the management of hypoxic-ischemic seizures in neonates undergoing therapeutic hypothermia. Dev Neurosci 2017; 39: 82-88.
[22] Tekgul H, Yalaz M, Kutukculer N, Ozbek S, Kose T, Akisu M, Kultursay N and Gokben S. Value of biochemical markers for outcome in term infants with asphyxia. Pediatr Neurol 2004; 31: 326-332.
[23] Dani C, Poggi C, Fancelli C and Pratesi S. Changes in bilirubin in infants with hypoxic-ischemic encephalopathy. Eur J Pediatr 2018; 177: 1795-1801.
[24] Soubasi V, Mitsakis K, Sarafidis K, Griva M, Nakas CT and Drossou V. Early abnormal amplitude-integrated electroencephalography (aEEG) is associated with adverse short-term outcome in premature infants. Eur J Paediatr Neurol 2012; 16: 625-630.
[25] Celtik C, Acunaş B, Oner N and Pala O. Neuron-specific enolase as a marker of the severity and outcome of hypoxic ischemic encephalopathy. Brain Dev 2004; 26: 398-402.
[26] Luo Y, Li J, Zhang J and Xu Y. Elevated bilirubin after acute ischemic stroke linked to the stroke severity. Int J Dev Neurosci 2013; 31: 634-638.
[27] Zhang XH, Zhang BL, Guo SM, Wang P and Yang JW. Clinical significance of dynamic measurements of seric TNF-α, HMGBl, and NSE levels and aEEG monitoring in neonatal asphyxia. Eur Rev Med Pharmacol Sci 2017; 21: 4333-4339.
[28] Ren XL, Li JH, Peng C, Chen H, Wang HX, Wei XL and Cheng QH. Effects of ABCDE bundle on hemodynamics in patients on mechanical ventilation. Med Sci Monit 2017; 23: 4650-4656.
[29] Tal S, Hadanny A, Sasson E, Suzin G and Efrati S. Hyperbaric oxygen therapy can induce angiogenesis and regeneration of nerve fibers in traumatic brain injury patients. Front Hum Neurosci 2017; 11: 508.
[30] Liu Z, Xiong T and Meads C. Clinical effectiveness of treatment with hyperbaric oxygen for neonatal hypoxic-ischaemic encephalopathy: systematic review of Chinese literature. BMJ 2006; 333: 374.
[31] Zhou BY, Lu GJ, Huang YQ, Ye ZZ and Han YK. Efficacy of hyperbaric oxygen therapy under different pressures on neonatal hypoxic-isch-

emic encephalopathy. Zhongguo Dang Dai Er Ke Za Zhi 2008; 10: 133-135.
[32] Azzopardi D; TOBY study group. Predictive value of the amplitude integrated EEG in infants with hypoxic ischaemic encephalopathy: data from a randomised trial of therapeutic hypothermia. Arch Dis Child Fetal Neonatal Ed 2014; 99: F80-F82.
[33] Sun J, Li J, Cheng G, Sha B and Zhou W. Effects of hypothermia on NSE and S-100 protein levels in CSF in neonates following hypoxic/ischaemic brain damage. Acta Paediatr 2012; 101: e316-e320.
[34] Mehta A, Kochanek PM, Tylerkabara E, Adelson PD, Wisniewski SR, Berger RP, Sidoni MD, Bell RL, Clark RS and Bell MJ. Relationship of intracranial pressure and cerebral perfusion pressure with outcome in young children after severe traumatic brain injury. Dev Neurosci 2010; 32: 413-419.
[35] Jia W, Lei X, Dong W and Li Q. Benefits of starting hypothermia treatment within 6 h vs. 6-12 h in newborns with moderate neonatal hypoxic-ischemic encephalopathy. BMC Pediatr 2018; 18: 50.
[36] Xue F, Huang JW, Ding PY, Zang HG, Kou ZJ, Li T, Fan J, Peng ZW and Yan WJ. Nrf2/antioxidant defense pathway is involved in the neuroprotective effects of Sirt1 against focal cerebral ischemia in rats after hyperbaric oxygen preconditioning. Behav Brain Res 2016; 309: 1-8.