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Study on the correlation of changes of IGF-1, GH, and NGB levels and NBNA score in neonates with hypoxic ischemic encephalopathy

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Abstract. – OBJECTIVE: The aim of this work was to investigate the correlation between dynamic changes of serum insulin-like growth factor 1 (IGF-1), growth hormone (GH), neuroglobin (NGB), and neonatal behavioral neurological assessment (NBNA) scores in different periods in neonates with hypoxic ischemic encephalopathy (HIE).

PATIENTS AND METHODS: Sixty HIE patients in the Neonatal Department of our hospital were selected. They were divided into the mild group (35 cases), moderate group (19 cases), and severe group (six cases) according to the diagnostic criteria. During the same period, 18 neonatal patients born at term in our hospital were chosen as the control group. Data were analyzed by SPSS19.0 statistical software (SPSS Inc., Chicago, IL, USA). The dynamic changes of IGF-1, GH, and NGB in different periods, as well as NBNA scores in HIE patients and in the control group, were analyzed. Furthermore, we analyzed the degree of correlation of IGF-1, GH, and NGB in different periods as well as NBNA scores in HIE patients and in the control group.

RESULTS: 1- IGF-1 levels between the three groups of HIE patients and control group had significant differences ($p<0.05$); 2- Comparing GH levels between the HIE experimental groups and control group, there was no statistical significance; 3-Comparing serum NGB levels between the three HIE experimental groups and control group, there were significant differences ($p<0.05$); 4- Comparing NBNA scores of the three groups of HIE patients and control group, there was a significant difference between the mild group and control group; 5- Serum IGF-1 and NBNA scores were positively correlated in the acute and recovery phase, while NGB level and NBNA scores were negatively correlated in the acute and recovery phase ($p<0.05$), which had statistical significance.

CONCLUSIONS: In neonatal HIE, serum IGF-1, GH, and NGB levels change. IGF-1 and NGB levels correlate with the prognosis of HIE.

Key Words

Hypoxic-ischemic encephalopathy (HIE), IGF-1, GH, NGB, NBNA.

Introduction

Neonatal hypoxic-ischemic encephalopathy (HIE) has several causes. The most common causes are abnormal amniotic fluid, hypoxia caused by extrusion in the process of childbirth, and coiling of the umbilical cord around the neck. These result in hypoxia-ischemia of brain tissue[1], which can result in corresponding damage and changes. Compared with premature infants, term infants often have higher incidence rates, generally characterized by: increased or reduced fetal heart rate, amniotic fluid severely contaminated by meconium, the existence of abnormal manifestations when rescue and recovery were performed after birth, such as action behaviors (shaking or active embrace reflex), muscular tension or primitive reflexes, central respiratory failure, and convulsion[2]. According to its severity, HIE is divided into mild, moderate, or severe. Mild is characterized primarily by nervous excitation, moderate by symptom inhibition (inhibited state, sleepiness or shallow coma, and decreased muscle tone), and severe by coma[3]. Among all body tissues, brain tissue metabolism is the most active. Therefore, hypoxia-ischemia frequently leads to damage of the nervous system, which can cause dysgnosia and cerebral palsy in neonates to a great extent. Neonatal behavioral neurological assessment (NBNA) can be used to comprehensively assess the nervous system and can reveal the degree of brain damage to HIE patients to some degree[4]. As a human growth hormone, insulin-like growth factor 1 (IGF-1) is an active polypeptide that plays an important role in proliferation and regulation of the nervous system. Growth hormone (GH) in serum also affects the expression and secretion of IGF-1. Studies have shown that GH affects the development of the neonatal nervous system. As a globulin, neuroglobin (NGB) can efficiently bind oxygen molecules and increase the oxygen utilization rate of brain tissue[5].

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The aim of the present work was to evaluate the relationship between changes of IGF-1, GH, and NGB levels in neonates with HIE, NBNA scores and disease severity.

Patients and Methods

Patients

Sixty patients with HIE who were hospitalized in the Neonatology Department of Xuzhou Children's Hospital from October 2011 to April 2013 were included in this study (32 males and 28 females). According to the diagnostic criteria, the patients were divided into three groups; mild, moderate, and severe group (Table I). There were 35 mild cases, 19 moderate cases, and six severe cases. Additionally, 18 neonatal patients born at full term in the Obstetrical Department of our hospital during the same period were selected as the control group, including 10 males and eight females.

Inclusion criteria: gestational age was from 37-42 weeks; the birth weight of all neonates in the experimental group and control group was from 2,500-4,000 g; all pediatric HIE patients met the diagnostic criteria and were diagnosed as mild, moderate, or severe cases; the general condition of the mother was good; subjects had no congenital malformations or metabolic diseases; all sample collections and evaluations of NBNA score were conducted with informed consent and agreement. Exclusion criteria: patients who died or whose parents expressed the desire to reject the continuation of the study; congenital brain abnormalities or convulsions caused by metabolic diseases; patients with parents with history of taking drugs, or present or long-term smoking; patients who did not meet the inclusion criteria. The approval for the study was obtained from the Ethics Committee of the Xuzhou Children's Hospital. Written informed consent was obtained from the legal guardians.

Sample Collection

Serum samples from all HIE patients in the experimental group were collected within 1 day (acute phase) and 14 days (recovery phase) after birth. Serum was collected from children in the control group on the first day after birth. A total of 3 ml femoral venous blood was drawn and slowly injected into non-anticoagulant vacuum blood tubes. Following incubation for 20 min at 28-30°C, samples were centrifuged at 1000 × g for 5-10 min. The upper layer of serum was stored in Eppendorf (EP) tubes at -90°C until use. Serum was used to measure the levels of IGF-1, GH, and NGB.

Measurement of IGF-1

Serum IGF-1 levels were measured by ABC-ELISA, also known as double antibody sandwich method. The kit was from R&D Corporation (Minneapolis, MN, USA). First, an IGF-1 single antibody was used to coat the enzyme-labeled plate. While adding anti-human IGF-1, IGF-1 was allowed to bind with the single antibody, including that from samples and standards. The formation of the immune complex and the enzyme-labeled plate are connected with each other, and the reaction color is changed by optical path difference (OPD) as the reaction enzyme. Solutions were presented as yellow and sulfuric acid solution was used as stop buffer. The optical density (OD) value at 490 nm was measured. The independent variable was the concentration of the

Table I. HIE scale division.

Scale division	Mild	Moderate	Severe
Consciousness	Hyperexcitation	Somnolent state	Coma
Moro reflex	More active	Incomplete	Disappeared
Sucking reflex	Normal	Weakened	Disappeared
Convulsion state	Accidental myoclonus	Frequently	Frequently occurred
Muscular tension	Normal	Decreased	Weakened
Pupil changes	Normal or enlarged	Shrunk, being slow to responding to light	Absent light reflex
Central respiratory failure	None	Yes	Severe
Bregma tension	Normal	Normal or slightly plump	Plump
Patient's condition and prognosis	In general, course of disease <3 days and the prognosis is good	<14 days, with sequelae	Continuing for several weeks, with high death rate

IGF-1 standard solution and the dependent variable was OD 490 nm. The concentration of IGF-1 in samples was calculated with reference to a standard curve. Standard curves were constructed as follows. Ten standard wells were used, 100 µl for each well. A total of 100 µl of diluted standard solution was placed in the first well. Next, 100 µl of solution from the first well was placed in the second well after being mixed evenly. This method was repeated to exponentially dilute solutions to the 9th well. Finally, 100 µl liquid from the 9th well was discarded so that each well contained 100 µl. The 10th well was used as the control well and the details are as follows. 1- a total of 50 µl solution was added to each well, including 50 µl diluted standards and 50 µl of the sample to be tested. After being mixed, they were incubated at 37-38°C for roughly 30 min; 2- the reaction plate was washed five times with washing solution, and then dried; 3- a total of 50 µl of the first antibody solution was added to each well and placed at 37-38°C for 30 min; 4- washing was performed again; 5- a total of 100 µl substrate working liquid was added to each well. After incubation at 38°C for 10 min, sulfuric acid solution was added to each well as stop buffer. After being mixed evenly, light absorption at 490 nm was measured; 6- the OD standard curve was constructed. The levels of IGF-1 were measured with reference to the standard curve.

Measurement of GH

Serum GH levels were measured by radioimmunoassay and the kit was purchased from Biofuge prime Heraeus (Porton Down, UK), with the determination range of 1-100 µg/l. After being mixed evenly at room temperature, 100 µl ^{130}IGH antibody and 100 µl serum were incubated for 24-30 h. Next, 500 µl immune separating agent was added and mixed evenly. After 20 min of incubation, samples were centrifuged at 4,000 rpm for 15 min and the supernatant was collected. The emanation coefficients of all tubes were automatically analyzed by a computer provided by Beijing Keya Biotechnology to calculate the results (Beijing, China).

Measurement of NGB

Serum NGB levels were measured by ELISA. The reaction was carried out at room temperature. The steps were as follows: 1- diluted NGB monoclonal antibody was coated on 100 wells of the enzyme-labeled plate. A total of 100 µl was added to each well and incubated at 5°C for 24 h; 2- standard plates were thoroughly washed with wash buffer five times; 3- each well was treated with 150 µl blocking solution. After incubation at room temperature for 5 h, washing was conducted again; 4- deionized water was used to exponentially dilute NGB standard samples. A total of 50 µl standards or 50 µl samples to be tested were added to appropriate wells. A total of 100 µl-deionized water was added to the last well as the blank control group. Each sample was analyzed in multiple wells; 5- after incubation at 37°C for 1 h, washing was conducted again; 6- after that 100 µl HRP-conjugated goat anti-rabbit IgG were added, plates were incubated at 37°C for 1 h; 7- after additional washing, 100 µl substrate buffer solution was added and incubated at room temperature while being shielded from light for 20 min; 8- sulfuric acid solution was added to each well as stop buffer. The OD standard curve was constructed. The independent variable was the concentration of NGB standard solution and the dependent variable was OD 450 nm. The serum concentration of NGB was calculated with reference to a standard curve.

Determination of Neonatal Nervous Behaviors

Physicians from the Department of Children Health in our hospital, through professional training, evaluated NBNA scores for the HIE experimental group and control group, including general condition, action behavior, muscular tension, and primitive reflex. Each parameter was assigned 0-2 points. All parameters were evaluated before sample collection, given the consent of family members was obtained. The peripheral environment was kept quiet and the temperature was maintained at a suitable level. The room temperature was maintained at about 26°C. All evaluation parameters were conducted in sleeping children at 1 h after being fed (Table II).

Statistical Analysis

SPSS18.0 statistical software (SPSS Inc., Chicago, IL, USA) was used for data analysis. All data are presented as mean ± standard deviation ($X \pm s$). X^2 test was applied for enumeration data. Pearson test was applied to compare and analyze the correlation between constants and variables. $p<0.05$ was considered statistically significant.

Table II. Relevant parameters of NBNA scores.

Parameter	General state	Scores		
		0 points	1 point	2 points
General situation				
Crying	Crying	None	Weak	Normal
Range of motion	Activity awakening	Excessive	Decreased	Normal
Degree of awakening	Awakening	Coma	Somnolence	Normal
Action behavior				
Formation of sound habit	In sleep	>10 times	6–10 times	<6 times
Formation of light habit	In sleep	>10 times	6–10 times	<6 times
Sensitivity for the face of speaker	Awakening	No eyeball reaction	Oculogyration<50°	Oculogyration>50°
Response to red ball	Awakening	No eyeball reaction	Oculogyration<50°	Oculogyration>50°
Coaxing and embracing	Crying	Cannot	Difficult	Easy
Primitive reflex				
Sucking reflex	Awakening	None	Weaker	Better
Embrace reflex	Awakening	None	Weaker	Better
Stepping reflex	Awakening	None	Weaker	Better
Muscular tension				
Traction reaction	Awakening	None	Some parts of body could be lifted	All limbs could be lifted
Straight hold reaction	Awakening	None	Supporting some parts of body	Supporting the whole body
Hand prehension	Awakening	None	Weaker	Better
Erect head	Awakening	None	More difficult	Easy
Scarf sign	Awakening	Worn around the neck	Elbow slightly exceeds median	Elbow fails to reach median
Forearm back rebound	Awakening	None	Weaker	Active
Lower limb rebound	Awakening	None	Slow and weak	Fast

Results

Comparison of General Conditions Between the HIE Experimental Group and Control Group

The experimental group included 60 cases of HIE who were divided into mild, moderate, and severe according to disease severity, including 35 mild cases, 19 moderate cases, and six severe cases. The control group included 18 healthy neonates, including 10 males and eight females. Comparing general parameters between the experimental group and control group, there were no significant differences in gestational age, birth weight, delivery mode, or gender (p>0.05) (Table III).

Table III. Comparison of general parameters between the experimental group and control group.

Group	Cases	Gestational age (W)	Birth weight (g)	Delivery mode		Gender	
				Cesarean section	Eutocia	Male	Female
Control group	18	39.44±1.33	3541.49±321.01	7	11	10	8
Mild HIE	35	39.25±1.41	3536.48±350.11	15	20	19	16
Moderate HIE	19	39.62±1.59	3349.27±381.01	9	10	11	8
Severe HIE	6	39.19±1.78	3391.35±430.82	2	4	3	3
F/χ^2		1.434	1.196	1.63		2.01	
p		>0.5	>0.5	>0.5		>0.5	

Note: p>0.05, the difference had no statistical significance.

Table IV. Comparison of serum IGF-1 levels of all HIE groups in the acute and recovery phase.

Group	Cases	IGF-1 level	
		Acute phase	Recovery phase
Control group	18	53.36±6.51	60.142±5.82
Mild HIE	35	36.12±2.49	58.26±6.4
Moderate HIE	19	29.22±3.68	41.39±7.0
Severe HIE	6	18.73±3.01	24.15±4.56
F/χ^2		8.53	7.26
p		<0.05	<0.05

Note: $p<0.05$, the difference had statistical significance.

Table V. Comparison of serum GH levels in all HIE groups in the acute and recovery phase.

Group	Cases	GH level	
		Acute phase	Recovery phase
Control group	18	28.81±5.32	11.03±3.98
Mild HIE	35	29.16±4.99	11.21±3.67
Moderate HIE	19	19.87±3.05	8.37±2.13
Severe HIE	6	8.56±4.43	5.76±4.19
F/χ^2		2.212	1.891
p		>0.05	>0.05

Note: $p>0.05$, the difference had no statistical significance.

Comparison of Serum IGF-1 Levels of all HIE Groups in the Acute and Recovery Phase

Serum IGF-1 levels of the mild, moderate, and severe HIE groups and control group in the acute and recovery phase were compared. The levels in all groups had significant differences. In the recovery phase, the IGF-1 levels in the control group and mild group were compared, and the difference had no statistical significance. However, there were evident differences between the control group, moderate group, and severe group; ($p<0.05$). The IGF-1 levels in the mild group were higher than in the moderate group. The IGF-1 levels in the moderate group were higher than in the severe group. The IGF-1 levels in the severe group were lower than in the other three groups. These data demonstrate that IGF-1 levels differ significantly with the severity of the patient's condition. Condition of increased severity was associated with lower IGF-1 level (Table IV and Figure 1).

Comparison of Serum GH Levels in all HIE Groups in the Acute and Recovery Phase

Serum GH levels in the mild, moderate, and severe HIE groups in the acute and recovery phase were compared, and there were no significant differences ($p>0.05$) (Table V and Figure 2).

Comparison of Serum NGB Levels in all HIE Groups in the Acute and Recovery Phase

In the acute and recovery phase, the serum NGB levels in all HIE groups and the control group in the acute phase were compared, and there were significant differences. The serum NGB levels between the mild group and moderate group, between the moderate group and severe group, and between the mild group and severe group were compared, and the differences were statistically significant ($p<0.05$) (Table VI and Figure 3).

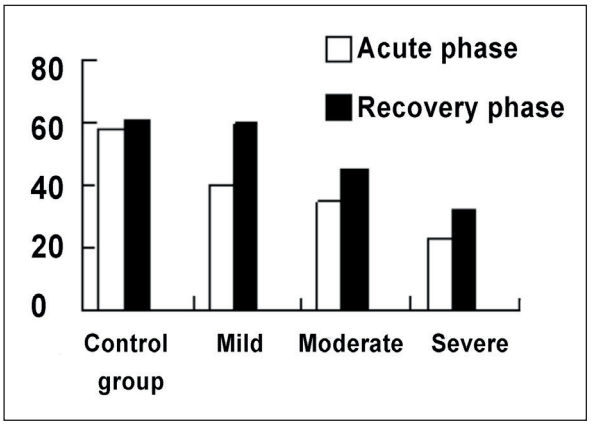

Figure 1. Comparison of IGF-1 levels in serum of all HIE groups in the acute and recovery phase.

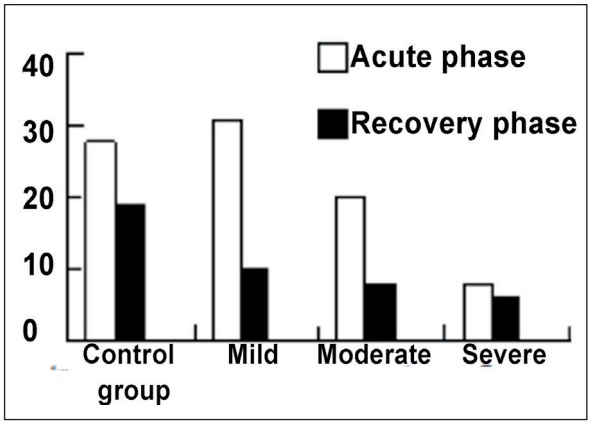

Figure 2. Comparison of GH levels in serum of all HIE groups in the acute and recovery phase.

Table VI. Comparison of serum NGB levels in all HIE groups in the acute and recovery phase.

Group	Cases	NGB level	
		Acute phase	Recovery phase
Control group	18	79.56±3.21	26.37±3.88
Mild HIE	35	88.63±4.91	29.76±5.35
Moderate HIE	19	182.36±6.22	95.35±5.76
Severe HIE	6	285.32±4.16	163.75±6.23
F/χ^2		9.27	8.63
p		<0.05	<0.05

Note: p>0.05, the difference had statistical significance.

Comparison of NBNA Scores in all HIE Groups in the Acute and Recovery Phase

The NBNA scores in the mild, moderate, and severe HIE groups were compared with those of the control group. There was no obvious difference between the mild group and control group. There were significant differences between the mild group, moderate group, and severe group; between the moderate group, control group, mild group, and severe group; and between the severe group, control group, mild group, and moderate group (p<0.05) (Figure 4).

Analysis of the Correlation Between Serum IGF-1, GH, and NGB Levels in HIE Patients and NBNA Scores

IGF-1 in serum from the mild, moderate, and severe HIE groups were positively correlated with NBNA scores (r=0.892, p<0.05, r=0.910, p<0.05). This shows that increased severity of the patient's condition was associated with lower IGF-1 level and lower NBNA score. However, the NGB level and NBNA scores showed a negative correlation in the acute and recovery phase (r=-0.63, p<0.05, r=-0.71, p<0.05), and there was statistical significance. GH level and NBNA scores had no correlation in the acute and recovery phase (Figures 5, 6, 7, 8).

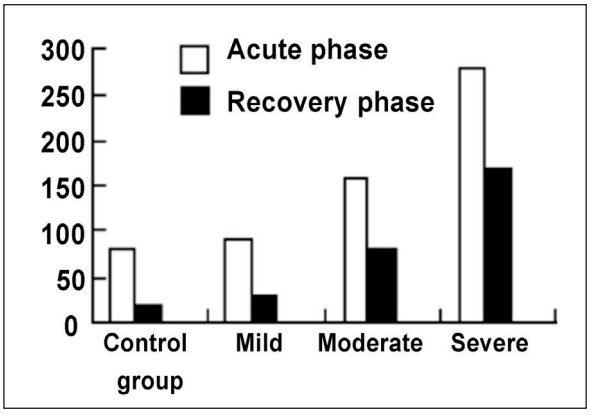

Figure 3. Comparison of NGB levels in serum of all HIE groups in the acute and recovery phase.

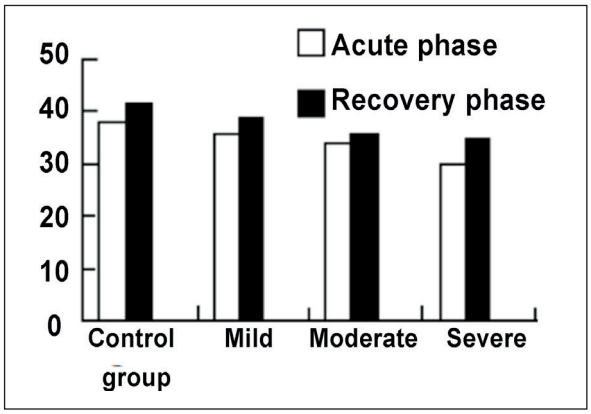

Figure 4. Comparison of NBNA scores of all HIE groups in the acute and recovery phase.

Discussion

Clinically, the anoxia of brain tissue is caused by various factors such as toxicity, asphyxia, or in utero wrapping of the cord around the neck causes HIE[6]. Moderate HIE can cause cerebral palsy and disorders of mental development to a great extent, which increases the death rate and severely lowers the quality of life of pediatric patients[7]. If hypoxia-ischemia in children can be identified as early as possible, and proper measures are applied for treatment and intervention, the asphyxia rate and death rate at birth and in the perinatal period can be effectively lowered, which has irreplaceable significance for improving quality of life[8]. IGF-1 is a regulatory factor of the nervous system with polypeptide composition[9]. With regard to the association of the condition of patients with HIE, there is a correlation, especially with the growth speed and degree of differentiation of neonatal nerve cells, which indirectly shows that it can promote the generation of new cells, tissues, and vessels, and lower the occurrence rate of death of cells of the nervous system[10]. When neonates have hypoxia-ischemia *in utero* or at birth, IGF-1 can play a significant role in protecting the nervous system. IGF-1 is polypeptide hormone generated and secreted by liver cells[11].

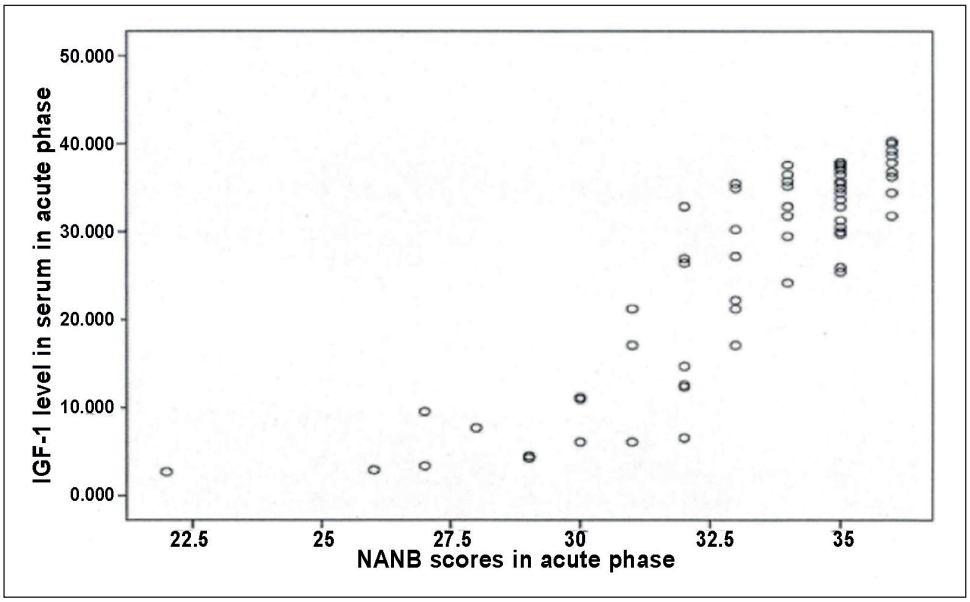

Figure 5. Correlation between serum IGF-1 level and NBNA scores in the acute phase.

Relevant researches showed that IGF-1 can protect the nervous system of children with HIE by different mechanisms, including lowering the pressure of neonatal brain blood vessels and decreasing the death rate of brain cells[12]. When newborns have HIE, the body automatically generates a response. IGF-1 in peripheral blood is transported to the brain cells of children via specific channels, thus effectively preventing the condition from progressing[13]. In this work, we compared serum IGF-1 levels in the HIE experimental groups and control group in the acute and recovery phase. There were significant differences between the levels in all groups. According to related studies, GH has a maintenance effect on nerve cells. Furthermore, GH affects the synthesis and secretion of IGF-1[14]. However, there is no established data showing whether it correlates with the severity of HIE and NBNA scores[15]. In this study, the relationship between serum GH level and the severity of HIE was not obtained from data analysis and it had no significant correlation with NBNA scores. NGB is a protein that functions in oxygen transport. A substantial amount of data has shown that

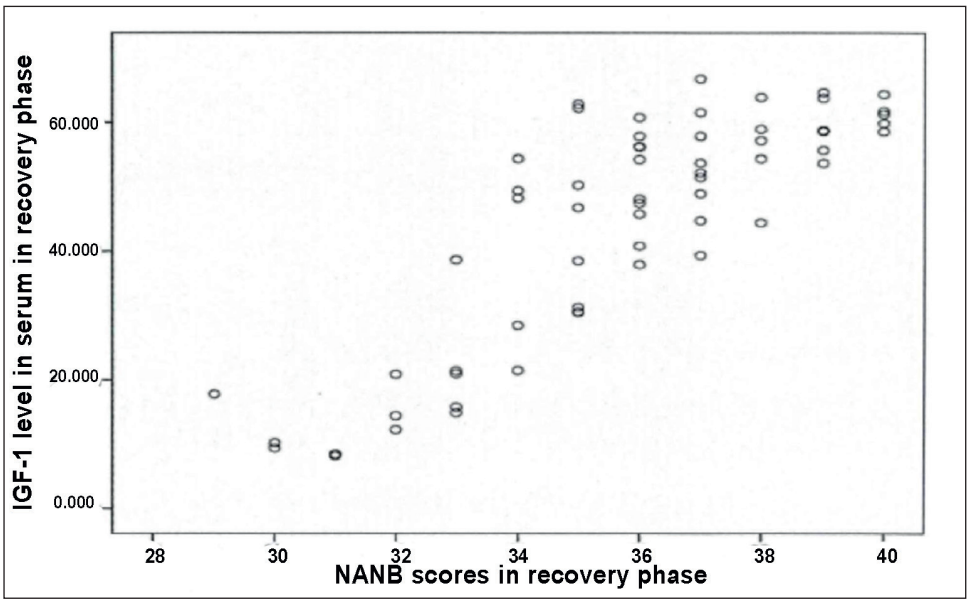

Figure 6. Correlation between serum IGF-1 level and NBNA scores in the recovery phase.

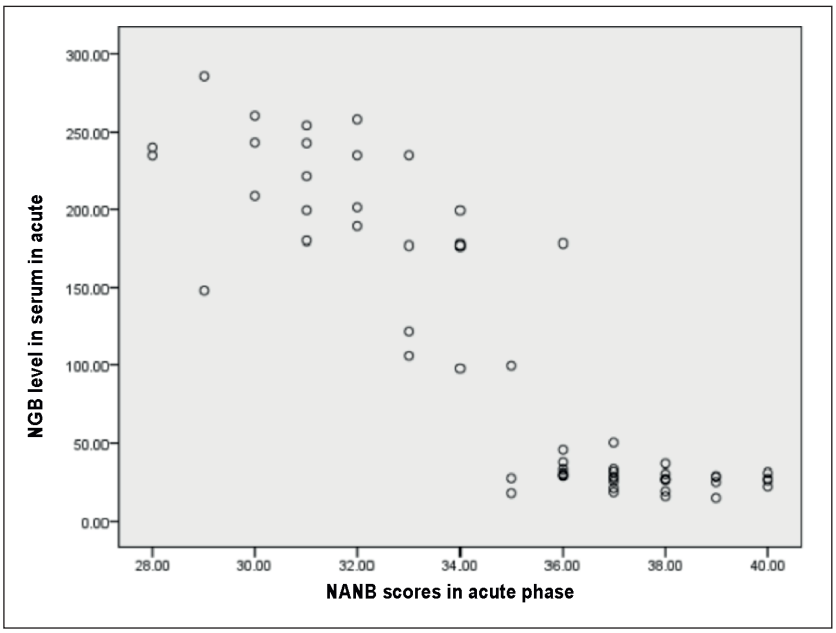

Figure 7. Correlation between serum NGB level and NBNA scores in the acute phase.

NGB can protect hypoxic-ischemic brain cells to a great extent[16]. First, NGB can reversibly bind oxygen molecules. Under the condition of hypoxia-ischemia, it can effectively bind oxygen with high affinity[17] to maintain the normal functions of the nervous system. When HIE occurs, the body can generate a series of toxic substances such as nitric oxide and carbonic oxide. NGB can effectively remove toxic substances and protect the nervous system[18]. This work showed that serum NGB levels in all of the HIE groups and control group had obvious differences. In addition, the NGB levels and NBNA scores showed a negative correlation in the acute and recovery phase.

Conclusions

HIE is an intricate disease. Clinically, specific cases of the disease should be comprehensively evaluated and analyzed by a combination of clinical manifestations, physical examination, laboratory examination, and imaging analysis of pediatric patients. Single indexes cannot be used

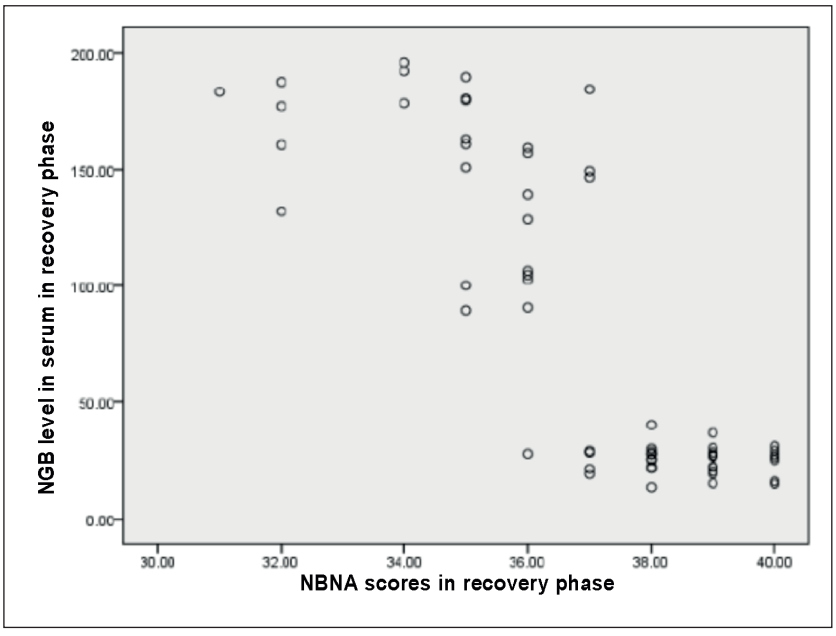

Figure 8. Correlation between serum NGB level and NBNA scores in the recovery phase.

to judge the severity and estimate the prognosis of a patient's condition[19]. To some extent, IGF-1 and NGB levels correlate with the condition and prognosis of HIE. They can be used to roughly judge the severity of the HIE patient's condition, although the severity requires further studies[20].

Conflict of Interest

The authors declared no conflict of interest.

References

1) Kurihara M, Shishido A, Yoshihashi M, Fujita H, Kohagizawa T, Ida H. [Long-term prognosis of children with hypoxic encephalopathy]. No To Hattatsu 2014; 46: 265-269.
2) Wang J, Tang Y, Zhang W, Zhao H, Wang R, Yan Y, Xu L, Li P. Insulin-like growth factor-1 secreted by brain microvascular endothelial cells attenuates neuron injury upon ischemia. FEBS J 2013; 280: 3658-3668.
3) Fletcher L, Isgor E, Sprague S, Williams LH, Alajajian BB, Jimenez DF, Digicaylioglu M. Spatial distribution of insulin-like growth factor binding protein-2 following hypoxic-ischemic injury. BMC Neurosci 2013; 14: 158.
4) Xu T, Li W, Liang Y, Yang Z, Liu J, Wang Y, Su N. Neuroprotective effects of electro acupuncture on hypoxic-ischemic encephalopathy in newborn rats Ass. Pak J Pharm Sci 2014; 27: 1991-2000.
5) Cotten CM, Murtha AP, Goldberg RN, Grotegut CA, Smith PB, Goldstein RF, Fisher KA, Gustafson KE, Waters-Pick B, Swamy GK, Rattray B, Tan S, Kurtzberg J. Feasibility of autologous cord blood cells for infants with hypoxic-ischemic encephalopathy. J Pediatr 2014; 164: 973-979.
6) Dang LT, Shavit JA, Singh RK, Joshi SM, Leber SM, Barks JD, Shellhaas RA. Subdural hemorrhages associated with antithrombotic therapy in infants with cerebral atrophy. Pediatrics 2014; 134: e889-e893.
7) Kracer B, Hintz SR, Van Meurs KP, Lee HC. Hypothermia therapy for neonatal hypoxic ischemic encephalopathy in the state of California. J Pediatr 2014; 165: 267-273.
8) Patel SD, Pierce L, Ciardiello AJ, Vannucci SJ. Neonatal encephalopathy: pre-clinical studies in neuroprotection. Biochem Soc Trans 2014; 42: 564-568.
9) Perez ME, Camprubi CM, Ramos CM, Anton LJ, Apodaca SA, Lopez RM, Garcia-Alix A. Treatment with bisphosphonates in severe hypercalcemia due to subcutaneous fat necrosis in an infant with hypoxic-ischemic encephalopathy. J Perinatol 2014; 34: 492-493.
10) Khan RH, Islam MS, Haque SA, Hossain MA, Islam MN, Khaleque MA, Chowdhury B, Chowdhury MA. Correlation between grades of intraventricular hemorrhage and severity of hypoxic ischemic encephalopathy in perinatal asphyxia. Mymensingh Med J 2014; 23: 7-12.
11) Shankaran S. Outcomes of hypoxic-ischemic encephalopathy in neonates treated with hypothermia. Clin Perinatol 2014; 41: 149-159.
12) Clark RH, Thomas P, Peabody J. Extrauterine growth restriction remains a serious problem in prematurely born neonates. Pediatrics 2003; 111: 986-990.
13) Agarwal KN, Gupta A, Pushkarna R, Bhargava SK, Faridi MM, Prabhu MK. Effects of massage & use of oil on growth, blood flow and sleep pattern in infants. Indian J Med Res 2000; 112: 212-217.
14) Field T. Massage therapy for infants and children. J Dev Behav Pediatr 1995; 16: 105-111.
15) Zhu XY, Ye MY, Zhang AM, Wang WD, Zeng F, Li JL, Fang F. Influence of one-year neurologic outcome of treatment on newborns with moderate and severe hypoxic-ischemic encephalopathy by rhuEP0 combined with ganglioside (GM1). Eur Rev Med Pharmacol Sci 2015; 19: 3955-3960.
16) Arnaiz SL, D'Amico G, Paglia N, Arismendi M, Basso N, del Rosario Lores Arnaiz M. Enriched environment, nitric oxide production and synaptic plasticity prevent the aging-dependent impairment of spatial cognition. Mol Aspects Med 2004; 25: 91-101.
17) Perlman JM. Neurobehavioral deficits in premature graduates of intensive care--potential medical and neonatal environmental risk factors. Pediatrics 2001; 108: 1339-1348.
18) Aicardi J. The etiology of developmental delay. Semin Pediatr Neurol 1998; 5: 15-20.
19) Blair C. Early intervention for low birth weight, preterm infants: the role of negative emotionality in the specification of effects. Dev Psychopathol 2002; 14: 311-332.
20) Beberashvili I, Sinuani I, Azar A, Kadoshi H, Shapiro G, Feldman L, Sandbank J, Averbukh Z. Decreased IGF-1 levels potentiate association of inflammation with all-cause and cardiovascular mortality in prevalent hemodialysis patients. Growth Horm IGF Res 2013; 23: 209-214.