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Original Article

Effect of gangliosides combined with mouse NGF on the expression of serum HIF-1α, NSE, and sICAM-1 levels in neonates with HIE

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Abstract: Objective: This study was intended to evaluate the effects of gangliosides combined with mouse nerve growth factor (NGF) on the expression of serum hypoxia-inducible factor-1α (HIF-1α), neuron-specific enolase (NSE), and soluble intercellular adhesion molecule-1 (sICAM-1) levels in neonates with ischemic-hypoxic encephalopathy (HIE). Methods: One hundred and thirty neonates with HIE admitted to our hospital from May 2017 to April 2019 were grouped into two groups according to the protocol of a randomized controlled trial, with 65 cases in each group. The control group received ganglioside treatment, while the combined group was treated with ganglioside + NGF for 2 weeks. Results: The total effective rate of treatment was higher in the combined group (90.77%) than in the control group (76.92%). The recovery time of sucking, consciousness, muscle tone and primitive reflexes was shorter in the combined group than in the control group, and the incidence of neurological sequelae was lower after 1 year in the combined group (4.62%) than in the control group (15.38%) ($P < 0.05$). Conclusion: Gangliosides combined with NGF can promote the recovery of muscle tone and reduce neurological sequelae, which may possibly be achieved by repairing damaged neuronal cells, enhancing antioxidant enzyme activity, and promoting the regression of inflammation.

Keywords: Neonatal ischemic-hypoxic encephalopathy, ganglioside, mouse nerve growth factor, HIF-1α, NSE

Introduction

Ischemic-hypoxic encephalopathy (HIE) may result in death, disability and chronic neurological injury in infants and children, mostly due to neuronal necrosis, edema and partial or complete hypoxia of brain cells induced by perinatal asphyxia, with clinical symptoms dominated by primitive reflex decline, poor muscle tone, absent sucking reflex and impaired consciousness [1-3]. If left untreated, as the condition continues to worsen, it can cause irreversible damage to the brain and induce permanent neurological sequelae such as epilepsy, mental retardation, and cerebral palsy, affecting the prognosis [4, 5]. The specific pathogenesis of HIE is still unclear, it involves neuronal apoptosis, brain neuronal cell metabolic disorders, cerebral hemodynamic changes, oxygen free radicals and other predisposing factors [6]. Due to the complex pathogenesis, there is no effective treatment option, and HIE is traditionally treated with antispasmodic therapy, intracranial pressure reduction, oxygenation, and cerebral neuroprotection, which can relive symptoms, but has little effect on damaged neuronal cells and neurological sequelae [7, 8]. Therefore, a safer and more effective treatment option for HIE has been the hot topic of neonatology research.

Gangliosides are a metabolic activator in brain cells, which can protect damaged brain cells, promote neuronal regeneration and neuronal growth, and inhibit brain cell apoptosis, thereby accelerating neurological recovery and reducing brain cell death [9]. Meta-analysis of a randomized controlled trial has shown that ganglio-

sides can improve neurological function in children with HIE, but the effect is far from satisfactory [10]. Mouse nerve growth factor (NGF) is an exogenous neurotrophic factor that functions after entering the lesion via the blood-brain barrier to repair damaged neurons, promote the growth of central neurons, scavenge oxygen free radicals, and improve neurological function [11]. However, few studies have explored the combination of the two drugs for the treatment of HIE. In this study, we observed the effects of gangliosides combined with NGF on the levels of hypoxia-inducible factor-1α (HIF-1α), neuron-specific enolase (NSE), and soluble intercellular adhesion molecule-1 (sICAM-1) in neonates with HIE.

Materials and methods

Clinical data

One hundred and thirty newborns with HIE treated in our hospital from May 2017 to April 2019 were included in this study, including 68 males and 62 females, with a mean gestational age of (38.68 ± 0.59) weeks, and were grouped into two groups according to the protocol of a randomized controlled trial, with 65 cases in each group. The control group received ganglioside treatment, while the combined group was treated with ganglioside + NGF for 2 weeks. Inclusion criteria: gestational age ≥ 37 weeks, birth weight ≥ 2500 g; HIE diagnostic criteria referred to the Diagnostic Criteria for Neonatal Hypoxic-Ischemic Encephalopathy [12], and the condition was clarified by CT, MRI and other imaging methods; all neonates received treatment in our NICU after birth; expected survival ≥ 15 d; the families of the neonates voluntarily signed the informed consent. Exclusion criteria: concomitant severe anemia or infection; inherited metabolic disorders or congenital malformations; intracranial hemorrhage caused by subarachnoid hemorrhage, intraventricular hemorrhage or parenchymal hemorrhage; coagulation disorders; concomitant systemic inflammatory response syndrome, central nervous system disease; hypersensitivity to drugs used in this study. This study was approved by the Ethics Committee of Qingdao Women and Children's Hospital.

Methods

(1) Basic treatment. Neonates in both groups received ECG monitoring, oxygenation, antispasmodic, intracranial pressure lowering, and fluid therapies in a warm environment. Energy support and improvement of microcirculation were also provided.

(2) Control group. I.v. infusion of q.d. 20 mg of ganglioside (Jilin Yinglian Biopharmaceutical Co., Ltd., H20093540, specification 2 mL: 20 mg) + 50 mL of 10% glucose injection was performed for a total of 2 weeks.

(3) Combined group. Ganglioside (administrated with the same method and dosage in the control group) + NSE treatment were provided. Thirty μg of NSE (Lizhu Group Lizhu Pharmaceutical Factory, S20100005, specification: 30 μg/bottle) was injected intramuscularly, q. d for 2 weeks.

Outcome measurement

(1) Therapeutic effect. According to the Diagnostic Criteria for Neonatal Hypoxic-Ischemic Encephalopathy, the efficacy criteria were determined as follows: neonates with stable breathing, clear consciousness, normal primitive reflexes and muscle tone, and reduction or termination of convulsions were considered as markedly effective. Neonates with stable breathing, significant improvement in consciousness, partial improvement of primitive reflexes and muscle tone, reduction of convulsions, and no convulsions after 5 days of treatment were considered effective. Neonates with unstable breathing, impaired consciousness, abnormal primitive reflexes or muscle tone, and occasional convulsions after 2 weeks of treatment were considered ineffective. Effective + markedly effective = total effective.

(2) Time to recovery of clinical symptoms. The time to recovery of sucking reflex, consciousness, muscle tone and primitive reflexes was recorded in both groups.

(3) Scale scores. The Neonate Behavior Nerve Assessment (NBNA) scale covers 5 parts, namely, behavior, active muscle tone, passive muscle tone, primitive reflexes, and general assessment, each scored with 0-2 points, totaling 40 points, with low scores indicating poor neurobehavior. A score greater than 37 points was defined as normal; the higher Mental Development Index (MDI) score and Psychomotor Development Index (PDI) score represented the better condition of development. A

Table 1. Comparison of general information between the two groups (n/X±S)

Group	Male/female	Gestational age (weeks)	Weight at birth (g)	Apgar score (points)	Severity of illness	Mode of delivery
					Mild/moderate/ severe	Natural birth/ cesarean section
Control group (n=65)	35/30	38.92±1.51	3096.54±124.15	6.56±1.21	26/25/14	20/45
Combined group (n=65)	33/32	38.53±1.66	3056.14±121.73	6.49±1.06	24/23/18	18/47
X^2/t	0.143	1.401	1.873	0.350	0.663	0.149
P	0.705	0.164	0.063	0.727	0.718	0.700

Table 2. Comparison of efficacy [n (%)]

Group	Markedly effective	Effective	Ineffective	Total effective
Control group (n=65)	15 (23.08)	35 (53.85)	15 (23.08)	50 (76.92)
Combined group (n=65)	23 (35.38)	36 (55.38)	6 (9.23)	59 (90.77)
X^2	-	-	-	4.600
P	-	-	-	0.032

score greater than 120 points, 70-120 points, and < 70 points represented excellent development, good development, and developmental defects, respectively. Assessments were made before and 2 weeks after treatment.

(4) Laboratory indices. Three mL of femoral venous blood was collected before and after 2 weeks of treatment, and the serum was separated by centrifugation (3000 r/min, R=8 cm) for 5 min at room temperature, and the supernatant was stored in -80°C. Enzyme-linked immunosorbent assay kits (Beijing Northern Institute of Biotechnology Co., Ltd.) was used to determine serum levels of glial fibrillary acidic protein (GFAP), myelin basic protein (MBP), sICAM-1, HIF-1α, lipoxin A4 (LXA4), endothelin-1 (ET-1), adrenomedullin (ADM); nerve peptide Y (NPY) levels were determined by radioimmunoassay (Shanghai Huzhen Industrial Co., Ltd.), and all operations were strictly in accordance with the kit instructions. NSE and 8-OHDG levels were determined by chemiluminescence method (Shanghai Jizhi Biochemical Technology Co., Ltd.)

(5) Neurological sequelae. The incidence of epilepsy, mental retardation, cerebral palsy, and ataxia were counted during one-year of follow-up.

Statistical analysis

With SPSS 24.0, the measurement data were expressed as $\bar{x} \pm s$, and the independent sample *t* and paired sample *t* tests were used for between-group and within-group comparisons, respectively. The count data were expressed as percentages and compared by χ^2 test. Graphpad prism 8.1 was adopted to produce statistical graphs. $P < 0.05$ was considered a significant difference.

Results

Baseline data

The baseline data of the combined group was not significantly different from the control group ($P > 0.05$), which showed that the two groups were comparable (**Table 1**).

Therapeutic efficacy

The total effective rate of treatment was 90.77% in the combined group and 76.92% in the control group. The total effective rate of treatment of the combined group was significantly higher than that of the control group ($P < 0.05$), indicating that ganglioside combined with NGF could effectively treat HIE (**Table 2**).

Recovery time of clinical symptoms

The recovery time of the sucking reflex, consciousness, muscle tone and primitive reflexes was shorter in the combined group than in the control group ($P < 0.05$), and the difference was statistically significant, indicating that ganglioside combined with NGF could accelerate the recovery of symptoms of HIE (**Figure 1**).

NBNA, MDI, and PDI scores

Before treatment, there was no significant difference in NBNA, MDI and PDI scores between

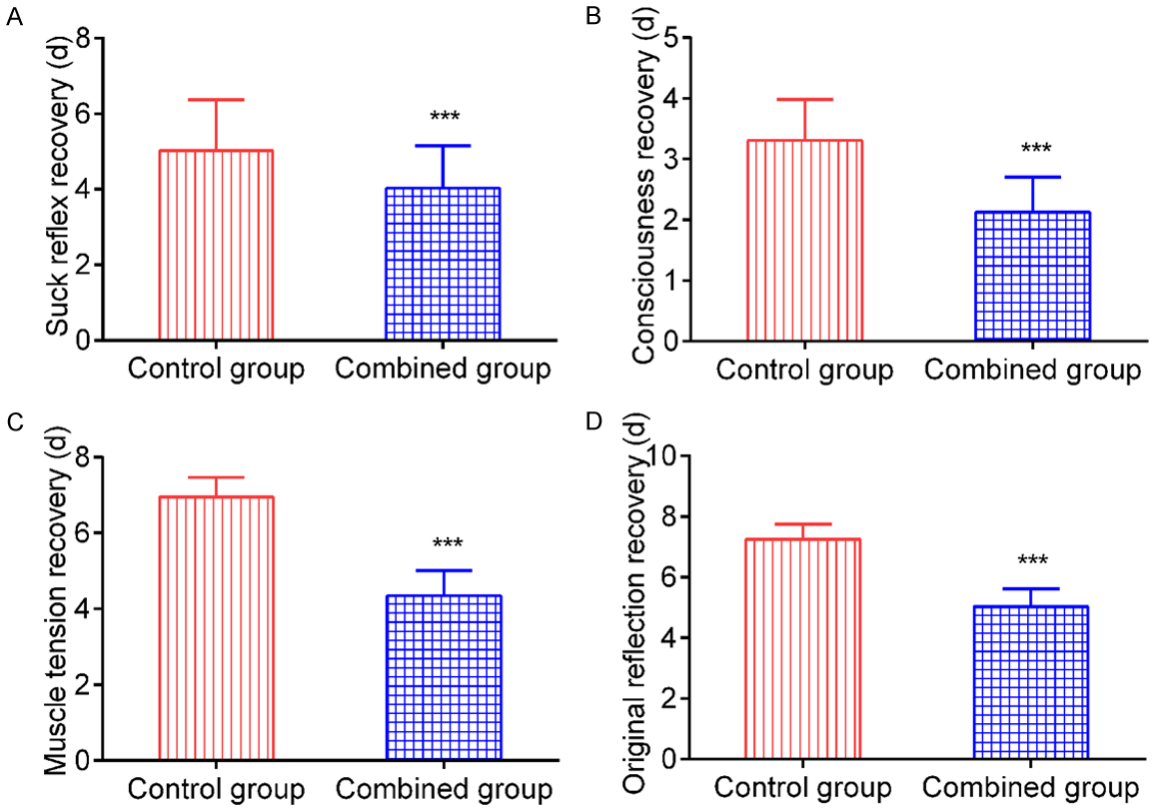

Figure 1. Comparison of recovery time (d). Note: (A) Recovery of sucking; (B) Recovery of consciousness; (C) Recovery of muscle tone; (D) Recovery of primitive reflexes. Compared to the control group, $^{***}P < 0.001$.

the two groups ($P > 0.05$). NBNA, MDI, and PDI scores were significantly higher in both groups after treatment ($P < 0.05$). After treatment, NBNA, MDI, and PDI scores were higher than those before treatment, and were higher in the combined group than in the control group ($P < 0.05$), indicating that ganglioside combined with NGF improved neurobehavioral, intellectual, and psychomotor development in infants with HIE (**Figure 2**).

Neurological function indicators

Before treatment, there was no significant difference in the levels of MBP, S100β, 8-OHDG, ADM and NPY between the two groups ($P > 0.05$). After treatment, the levels of MBP, S100β, 8-OHDG, ADM and NPY were significantly lower than those before treatment, showing significant difference ($P < 0.05$). The levels of MBP, S100β, 8-OHDG, ADM, and NPY were lower in the combined group than in the control group after treatment ($P < 0.05$), indicating that ganglioside combined with NGF could improve neurological function and repair neurons in infants with HIE (**Figure 3**).

Comparison of serum-related indices

The levels of HIF-1α, NSE, sICAM-1, ET-1 and GFAP had no significant difference between the two groups before treatment ($P > 0.05$). After treatment, the levels of HIF-1α, NSE, sICAM-1, ET-1 and GFAP in both groups were significantly lower than those before treatment ($P < 0.05$). The levels of HIF-1α, NSE, sICAM-1, ET-1, and GFAP were lower in the combined group than in the control group after treatment ($P < 0.05$), indicating that ganglioside combined with NGF could enhance immune function, improve vascular injury, promote inflammation regression, and scavenge free radicals in infants with HIE (**Figure 4**).

Neurological sequelae

The incidence of neurological sequelae was lower in the combined group (4.62%) than in the control group (15.38%) during the follow-up

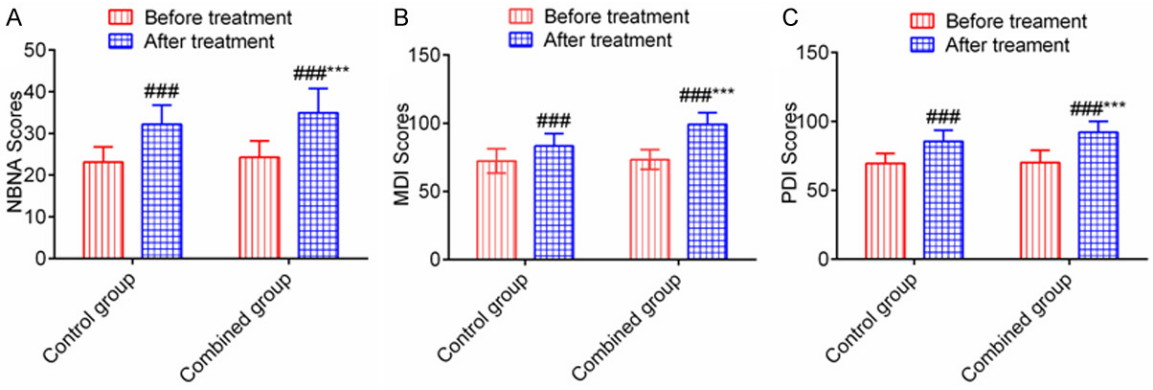

Figure 2. Comparison of NBNA, MDI, and PDI scores. Note: (A) NBNA; (B) MDI; (C) PDI. Compared to the control group, $^{***}P < 0.001$; compared to the pre-treatment within same group, $^{###}P < 0.001$.

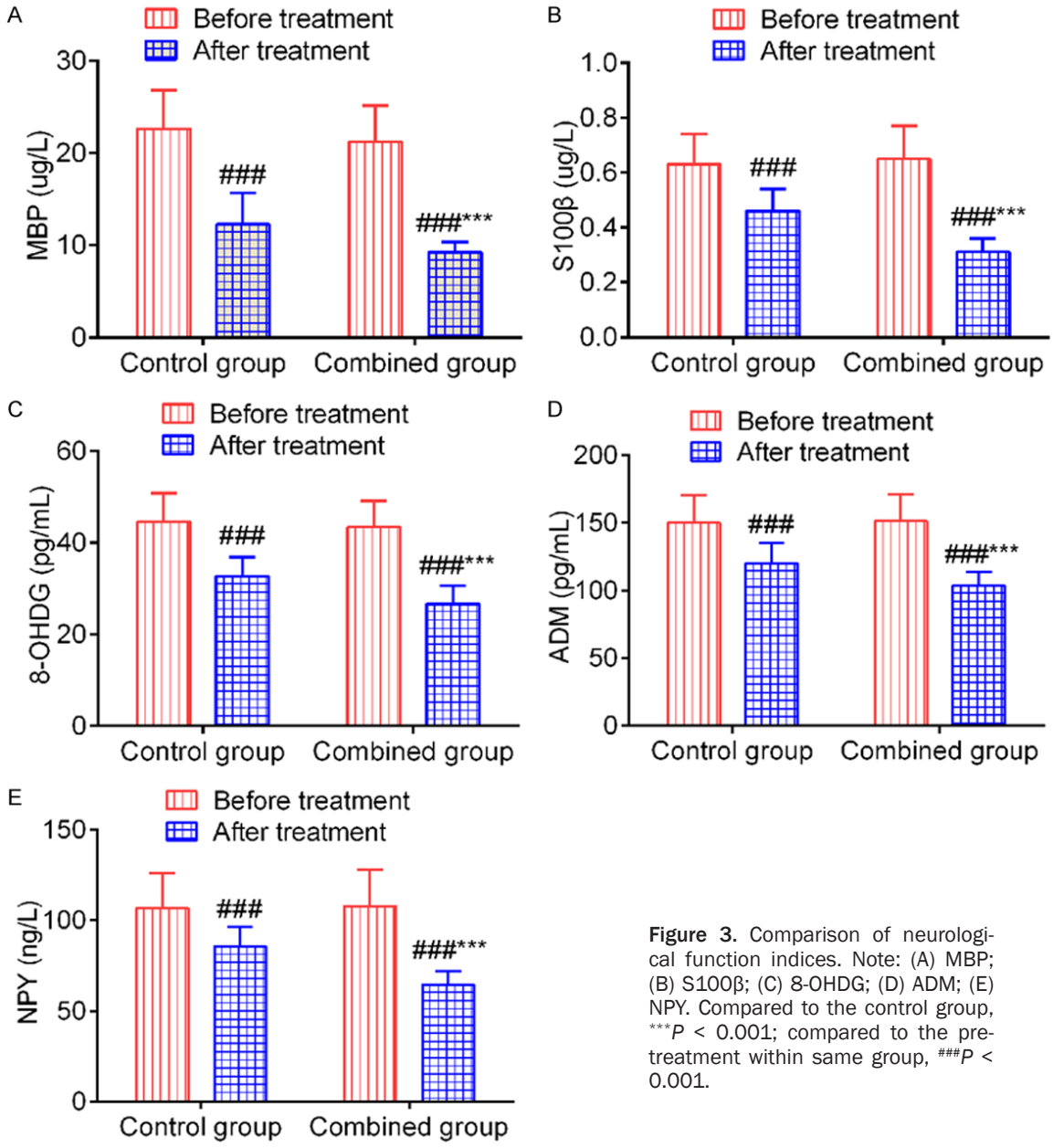

Figure 3. Comparison of neurological function indices. Note: (A) MBP; (B) S100β; (C) 8-OHDG; (D) ADM; (E) NPY. Compared to the control group, $^{***}P < 0.001$; compared to the pre-treatment within same group, $^{###}P < 0.001$.

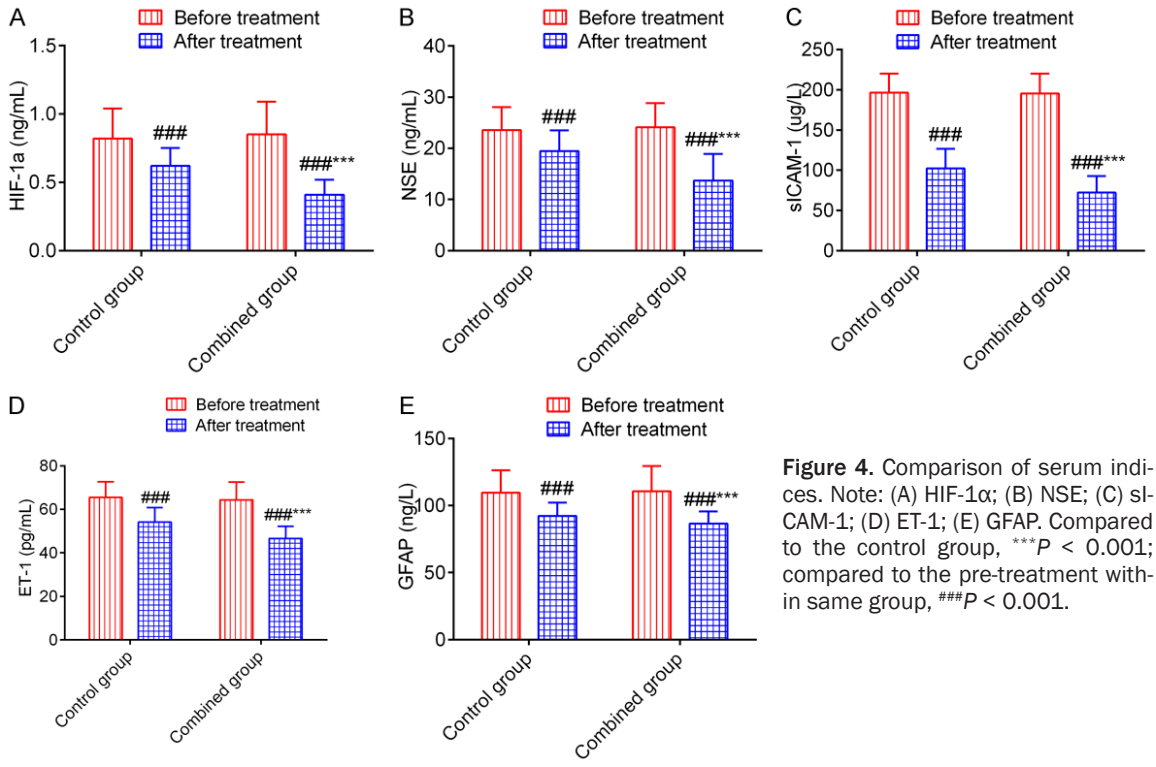

Figure 4. Comparison of serum indices. Note: (A) HIF-1α; (B) NSE; (C) sICAM-1; (D) ET-1; (E) GFAP. Compared to the control group, $^{***}P < 0.001$; compared to the pre-treatment within same group, $^{###}P < 0.001$.

Table 3. Comparison of neurological sequelae during one-year follow-up [n (%)]

Group	Seizures	Mental retardation	Cerebral Palsy	Ataxia	Total
Control group (n=65)	3 (4.62)	2 (3.08)	3 (4.62)	2 (3.08)	10 (15.38)
Combined group (n=65)	2 (3.08)	0 (0.00)	1 (1.54)	0 (0.00)	3 (4.62)
X^2	-	-	-	-	4.188
P	-	-	-	-	0.041

($P < 0.05$), indicating that ganglioside combined with NGF could reduce the risk of neurological sequelae (**Table 3**).

Discussion

Severe hypoxia in infants with HIE can cause neurological dysfunction and brain cell edema; meanwhile, vascular endothelial cells and brain neurons are damaged by oxygen free radicals and excitatory amino acids, increasing blood-brain barrier permeability and causing microcirculatory disorders in brain tissue, thus forming a vicious circle [13-15]. Therefore, neonatal HIE should be treated with inhibiting neuronal apoptosis, improving brain cell hypoxia, and repairing damaged neuronal cells.

The results of this study found that the total effective rate and post-treatment NBNA, MDI, and PDI scores were higher in the combined group than in the control group, the recovery time of sucking, consciousness, muscle tone, and primitive reflexes was shorter in the combined group than in the control group, and the incidence of neurological sequelae after 1 year was lower in the combined group than in the control group, indicating that ganglioside combined with NGF could effectively treat neonatal HIE, promote disappearance of clinical symptoms, and reduce neurological sequelae [16, 17]. In a meta-analysis, Zhao et al. [18] confirmed that the combination of NGF and gangliosides showed higher clinical efficiency, shorter time to recovery of consciousness, time to disappearance of convulsions, time to recovery of muscle tone, and time to recovery of reflexes compared with treatment with gangliosides alone, which were similar to the results of Luo [19], and Chou et al. [20]. The reason may be that gangliosides enter the cell membrane via

the blood-brain barrier and play an important role in protecting the functions of ion pumps, maintaining the activity of cell membrane ion gradients and adenosine triphosphatase, inhibiting intracellular calcium aggregation, maintaining intracellular ion homeostasis, and protecting nervous tissue. NGF enhances cellular stress responses, boosts antioxidant capacity and oxygen radical scavenging capacity of brain tissue [21, 22], maintains intracellular concentration of ionized calcium, and also activates cell survival signaling pathways, inhibits apoptotic proteins, antagonizes the neurotoxicity of excitatory amino acids, promotes axonal proliferation and growth, and induces neuronal regeneration [23, 24]. Therefore, gangliosides combined with NGF are involved in protecting brain function and repairing brain injury through multiple mechanisms.

Hypoxia and ischemia can promote the release of many inflammatory factors, cytokines and immune molecules such as sICAM-1, TNF-α and interleukins, which induce excessive inflammatory responses and immune system disorders in brain tissue. sICAM-1 is involved in immune responses and inflammatory responses *in vivo*, mediating epithelial endothelial cells and interleukocyte adhesion. After the hypoxic-ischemic reperfusion, sICAM-1 expression is upregulated, inducing a massive release of inflammatory mediators, TNF-α and interleukin-6 (IL-6), exacerbating the condition. HIF-1α expression is induced by hypoxia-ischemia, and when the body is in a hypoxic-ischemic state, hypoxia-inducible factors bind to the aggregated HIF-1α and form a complex that translocates to the nucleus, which initiates transcription of target genes. Lu et al. [25] found that in infants with HIE, a large amount of HIF-1α was induced by ischemia and hypoxia and released into the blood circulation, which reduced the oxygen uptake capacity of brain tissue and exacerbated the hypoxic state and brain tissue injury. NSE can be used as an indicator of central nervous system injury. When nerve cells are disintegrated, degenerated and damaged during brain injury, plasma NSE is released into the blood and cerebrospinal fluid, resulting in elevated NSE levels. Therefore, reducing HIF-1α, NSE, and sICAM-1 levels could improve symptoms of neonatal HIE. This study showed that the levels of HIF-1α, NSE, sICAM-1 were lower in the combined group than in the control group after treatment, confirming that ganglioside combined with NGF could enhance immune function, inhibit neuronal apoptosis and promote the recovery of central nervous function.

In conclusion, ganglioside combined with NGF was effective in treating HIE, promoting the recovery of muscle tone, improving neurobehavior, and reducing neurological sequelae, which may be related to repairing damaged neuronal cells, enhancing antioxidant enzyme activity, and promoting the regression of inflammation. However, the present study has shortcomings such as a small sample size, single source of patients, short follow-up time and failure to assess the safety of the drugs, which should be improved in future studies.

Disclosure of conflict of interest

None.

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