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Detection of CGRP in plasma and cerebrospinal fluid of neonatal hypoxic-ischemic encephalopathy and its clinical significance

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Abstract

Purpose: Neonatal hypoxic-ischemic encephalopathy (HIE) has a high incidence, disability and mortality rate. Calcitonin gene-related peptide (CGRP) exerts a protective role in brain injury. However, whether CGRP plays a role in HIE is unclear. Our study intends to measure CGRP level in HIE children.

Materials and Method: 93 neonates with HIE were selected as research subjects and 41 cases of neonates admitted to our hospital were selected as control group. The level of CGRP, calcium-binding protein (S-100β) and neuron-specific enolase (NSE), and NBNA score was measured.

Results: The levels of CGRP, S-100β, and NSE in plasma and cerebrospinal fluid of children with HIE were significantly elevated compared to controls ($P < 0.05$) and gradually increased with the elevated HIE severity ($P < 0.05$). The levels of CGRP, S-100β, and NSE in HIE children in recovery stage were significantly lower than those in acute stage ($P < 0.05$). CGRP levels were positively correlated with S-100β and NSE levels ($P < 0.05$); and negatively correlated with NBNA scores ($P < 0.05$).

Conclusions: Plasma and cerebrospinal fluid CGRP level have clinical significance in the early diagnosis of HIE and assessment of the disease severity.

Keywords: HIE; CGRP; plasma; cerebrospinal fluid.

Introduction

HIE is caused by a decrease in cerebral blood volume and partial or complete hypoxia in the perinatal asphyxia of neonates, causing severe brain injury syndrome [1]. The incidence of HIE in newborns is high, the condition is dangerous, the mortality rate is high with permanent neurological deficits remain in surviving children. Studies have shown that moderate and severe HIE can cause permanent neurological deficits and neurodevelopmental changes in 48% of children and can cause death in 27% of children [2], which seriously affects the quality of life of children [3]. Therefore, the early diagnosis and accurate assessment of the severity of the child's condition and reasonable treatment options have great significance to improve the quality of life. With the development and application of molecular biology, more and more researches are devoted to finding new and efficient biomarkers for the clinical diagnosis of HIE [4, 5]. FasL and IL-6 levels in cerebrospinal fluid are more predictive of the severity and long-term prognosis of infantile encephalopathy after asphyxia than standard biomarkers [6]; IL-10 and IL-8 levels in HIE children were significantly higher than normal children, and associated with the HIE severity. After mild hypothermia treatment, IL-10 and IL-8 serum levels were significantly reduced, suggesting that IL-10 and IL-8 are not only associated with the occurrence and development of HIE but also closely related to treatment efficacy [7]. However, the disease pathogenesis is a very complicated process, and several molecules are involved. The pathogenesis of HIE is still not completely clear, so the exploration of the pathogenesis needs further studies. CGRP is an active polypeptide mainly distributed in the nervous system, cardiovascular system and lung tissues. It has a strong vasodilating effect and can directly expand cerebral blood vessels during cerebral hypoxia, increase cerebral perfusion, and maintain intracellular Ca2 + homeostasis. It can also prevent the influx of Ca2 + into cells with a direct protective effect on brain cells [8]. Hypoxia is a joint factor in the development of HIE and can cause the

Running title: CGRP level in neonatal HIE and clinical significance
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impaired energy metabolism of brain cells, brain hemodynamic changes, and damage to the nervous system of the brain. Studies have found that CGRP can improve the inflammation and apoptosis of cells due to hypoxia [9]. Therefore, we speculated that CGRP might be involved in HIE development and there are few reports on the related research of CGRP in HIE. In our study, the levels of CGRP in cerebrospinal fluid and plasma of neonatal HIE were measured to explore whether it plays a role in neonatal HIE.

Materials and Method

Patients

93 cases of HIE neonates from January 1, 2016 to December 31, 2019 were selected as the research subjects. Acording to the clinical diagnosis and indexing standards of HIE developed by the Neonatal Group of the Chinese Medical Association, HIE neonates were divided into three groups: mild (35 cases), moderate (30 cases) and severe group (28 cases), and 41 cases of neonates hospitalized in the same period of our hospital were selected as the control group. The control group had no history of asphyxia and nervous system diseases and severe infections. The clinical data of newborns in each group were shown in Table 1. There were no statistical significances in the gender, gestational age, birth weight, and birth / cesarean section of each group of newborns, indicating that the newborns in each group were comparable. Inclusion criteria for study subjects: 1) All children with HIE who met the HIE clinical diagnosis and indexing standards formulated by the Neonatal Group of the Chinese Medical Association; 2) gestational age ≥ 37 weeks and neonatal weight ≥ 2000g; 3) No history of genetic diseases and infectious diseases; 4) the mother has no bad habits such as smoking and drinking, and there is no history of special medication during pregnancy. Exclusion criteria: 1) severe abnormal brain development; 2) intrauterine infection; 3) congenital malformations and suspected intracranial hemorrhage. All the study subjects and their families have signed informed consent and this study has been reviewed and approved by The Affiliated Hospital of Xianning Central Hospital ethics committee.

Neonatal behavioral neuroscoring

Neonatal behavioral neurological assessment (NBNA) scores were performed at 24 h after birth in each group of newborns using the "Chinese Neonatal Behavioral Neuroscore 20", including 3 general evaluations, 3 primitive reflexes, 4 active muscle strengths, 4 passive muscle tensions, and 6 behavioral abilities, each score has 3 points from 0 to 2. Scoring was performed by a professional doctor.

Specimen collection

Cerebrospinal fluid and venous blood were collected within 24 hours (acute phase) and 7 days (recovery period) after birth. The cerebrospinal fluid and venous blood were centrifuged at 3000 r / min for 10 to 15 min at 4 °C to collect the supernatant which was saved at -80 °C for subsequent detection.

Detection of CGRP, S-100β, NSE levels in plasma and cerebrospinal fluid by ELISA

After thawing plasma and cerebrospinal fluid specimens, CGRP (Shanghai Yuanmu Biotechnology Co., Ltd.), S- 100β (Shanghai Keshun Biotechnology Co., Ltd.), NSE (Shanghai Renjie Biotechnology Co., Ltd.) level was measured by ELISA kits in accordance with the kit instructions.

Statistical methods

SPSS 22.0 software was adopted for analyzing data which were displayed as mean ± standard deviation and assessed by t test or ANOVA. Correlation analysis of measurement data was assessed by Pearson correlation. $P < 0.05$ indicates a difference.

Results

Comparison of CGRP, S-100β and NSE levels in plasma

CGRP, S-100β, and NSE levels in the plasma of children in each group were measured by ELISA. As shown in Table 2, compared to controls, CGRP, S-100β, and NSE levels in the mild, moderate, and severe groups of HIE were significantly increased ($P <0.05$). CGRP, S-100β, NSE plasma levels showed an upward trend with the increased severity of HIE ($P <0.05$). Their levels in children with HIE in the recovery period were significantly reduced compared to children in acute phase ($P <0.05$).

Comparison of CGRP, S-100β and NSE levels in cerebrospinal fluid

At the same time, the levels of CGRP, S-100β, and NSE in cerebrospinal fluid of children during the acute phase and recovery phase were also measured by ELISA. As shown in Table 3, significantly increased levels of CGRP, S-100β, and NSE were found in all groups of HIE ($P <0.05$). With the increased severity of HIE, the levels of CGRP, S-100β, and NSE cerebrospinal fluid showed an increasing trend ($P <0.05$). The levels of CGRP, S-100β, and NSE in HIE during the recovery stage were

significantly decreased compared to acute stage (P <0.05).

Correlation of CGRP levels with S-100β and NSE

The correlation between CGRP levels in plasma and cerebrospinal fluid with S-100β and NSE in HIE was analyzed by Pearson correlation and found that the level of CGRP in plasma was positively correlated with S-100β and NSE (r = 0.707, P <0.001; r = 0.770, P <0.001) (Figure 1); CGRP in cerebrospinal fluid and S-100β and NSE showed a significantly positive correlation (r = 0.640, P <0.001; r = 0.857, P <0.001).

Correlation of CGRP levels with NBNA scores

The NBNA score of each group of newborns was shown in Figure 2A. The control group was (38.41 ± 1.12) points, the mild group was (36.50 ± 1.38) points, the moderate group was (33.61 ± 1.84) points, and the severe group was (30.72 ± 1.78) points, the NBNA scores of the four groups of newborns were significantly different (F = 162.41, P <0.001). The NBNA scores of mild, moderate and severe groups of children with HIE were significantly reduced compared to controls (P <0.05). As the severity of HIE increased, the NBNA score decreased gradually ((P <0.05)).

As seen in Figures 2B and 2C, CGRP levels were negatively correlated with NBNA scores (r = -0.694, P <0.001; r = -0.731, P <0.001).

Discussion

HIE can cause neurological sequelae such as mental retardation, epilepsy, and cerebral palsy in neonates, which seriously affects the children's quality of life. At present, the diagnosis of HIE mainly depends on clinical symptoms and physical examination, and there is still lack of sensitive laboratory diagnostic indicators, so the best opportunity for treatment is lost. Although neurological function assessment and EEG can be used to evaluate the severity of children with HIE, they are susceptible to factors such as their mental state and physical health, and lack the necessary objectivity. Moreover, HIE is a complication after neonatal asphyxia and part of the ischemic brain tissue is not immediately necrotic. Therefore, the early diagnosis and treatment can prevent the nerve cell blood-bearing disorder from continuing to aggravate, and at the same time restore necrotic nerve tissue, which can reduce the lethality of HIE. The rate and disability rate play an important role, so it is of great significance to find biomarkers for the early diagnosis of HIE. CGRP can improve the hypoxia and apoptosis of cells, and there are few reports on its role in neonatal HIE. Therefore, this study assessed CGRP's clinical significance in neonatal HIE. We found that the levels of CGRP in children with HIE in plasma and cerebrospinal fluid were significantly elevated, and gradually increased with the elevated severity of HIE, and with significantly lower level in recovery stage than acute phase, suggesting that CGRP might be involved in the development and pathogenesis of HIE. The NBNA score is a commonly used clinical method for examining the nervous system of newborns. After analyzing the correlation between CGRP levels and NBNA scores, it was found that CGRP levels in plasma and cerebrospinal fluid showed a significantly negative correlation with NBNA, suggesting that CGRP might be a biomarker for assessing brain injury. At present, there are no reports about the role of CGRP in the pathogenesis of HIE. However, in the study of Du Z et al. [10], it was found that CGRP can inhibit astrocyte apoptosis and has a protective effect on cerebral ischemic injury in rats by regulating Wnt / β-Catenin signaling pathway; Zhai L et al. [11] reported that CGRP gene knockout mice had significantly higher levels of inflammatory cytokines in the acute phase of cerebral ischemic injury than wild-type mice. The results showed that endogenous CGRP had a protective effect on ischemic brain injury. We speculate that the increase in plasma CGRP level during the acute phase of cerebral ischemia and hypoxia is a self-protection mechanism, which is that when the blood-brain barrier is damaged, peripheral CGRP enters to the brain to participate in the pathological response of the body, expands the cerebral blood vessels, and plays a self-protective role in brain injury. Borkum J M. et al. [12] also found that stresses such as cerebral ischemia, injury, and fever can increase CGRP levels, activate anti-apoptotic signaling pathways, upregulate neurotrophic factors and reduce brain edema, thereby protecting brain damage. S-100β and NSE are indicators of early brain damage. Normally, their expression is low. When brain injury occurs, S-100β and NSE are released into the cerebrospinal fluid and cell spaces. Serum S-100β and NSE have been used in the evaluation of HIE [3, 5, 13] and researchers have used S-100β and NSE levels to evaluate the efficacy and prognosis of HIE [14-17] and can also be used to find new HIE markers [18]. We also tested S-100β and NSE levels to clarify the correlation between CGRP and S-100β and NSE and found significantly increased S-100β and NSE levels in HIE which were closely related to the severity of the disease, consistent with the results of previous studies [19, 20]. S-100β and NSE levels in cerebrospinal fluid are consistent with their expression in plasma, suggesting that the detection

of cerebrospinal fluid S-100β and NSE levels can also determine the occurrence and progression of HIE to a certain extent. Analysis of the correlation between CGRP and S-100β and NSE in plasma and cerebrospinal fluid found that CGRP levels were positively correlated with S-100β and NSE. This result further suggests that CGRP might be involved in HIE and a biomarker for HIE early diagnosis. However, the study on the diagnosis of HIE combined with CGRP, S-100β, and NSE has not been elaborated which is a main study limitation. Therefore, further research is needed in future research. In addition, the mechanism of the role of CGRP in the development of HIE has not been further analyzed in this study. Therefore, further study is required to provide more theoretical basis for the development of HIE and provide in-depth information for the application of CGRP in clinical diagnosis of HIE.

Disclosure of conflict of interest

None.

Conclusion

CGRP, S-100β, and NSE levels are abnormal in HIE. Plasma and cerebrospinal fluid CGRP have clinical significance in the HIE early diagnosis and severity assessment.

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Tables and Figure legends

Table 1. **Comparison of clinical data of each group.**

Group	n	Gender (M/F)	Gestational age (weeks)	Birth weight (g)	Abortion/cesarean section
Control	41	21/20	38.2±1.7	2801.6±403.6	27/14
Mild	35	19/16	38.4±1.6	2789.8±405.8	21/14
Moderate	30	15/15	38.3±1.8	2826.4±411.6	18/12
Severe	28	13/15	38.1±1.9	2768.2±409.5	16/12
P		0.94	0.91	0.96	0.89

Table 2. **Comparison of CGRP, S-100β, and NSE levels in the plasma of newborns in each group.**

		Acute phase			Recovery phase		
Group	n	CGRP (μg/L)	S-100β (μg/L)	NSE (μg/L)	CGRP (μg/L)	S-100β (μg/L)	NSE (μg/L)
Control	41	55.23±15.44	1.23±0.91	9.86±1.97			
Mild	35	69.45±16.56	2.15±0.86	21.42±2.36	56.12±13.21	1.43±0.89	14.67±2.84
Moderate	30	89.41±14.87	3.52±1.01	29.72±2.56	68.51±16.48	2.06±0.92	18.54±2.73
Severe	28	116.26±15.78	4.89±1.13	37.63±2.64	79.39±15.67	2.68±1.03	22.56±2.96
F	-	92.73	89.74	869.98	18.73	13.71	60.11
P	-	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Table 3. **Comparison of CGRP, S-100β, and NSE levels in cerebrospinal fluid of newborns in each group.**

		Acute phase			Recovery phase		
Group	n	CGRP (μg/L)	S-100β (μg/L)	NSE (μg/L)	CGRP (μg/L)	S-100β (μg/L)	NSE (μg/L)
Control	41	10.28±2.16	0.62±0.18	7.42±1.68			
Mild	35	17.56±2.78	0.78±0.16	13.74±1.72	10.39±2.57	0.66±0.17	9.76±1.82
Moderate	30	24.68±2.46	0.91±0.21	18.26±1.83	16.56±2.43	0.79±0.19	12.18±1.76
Severe	28	30.78±2.95	1.20±0.23	23.02±2.03	21.15±2.86	0.93±0.20	16.78±1.89
F	-	402.16	52.26	464.62	134.28	16.44	116.91
P	-	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

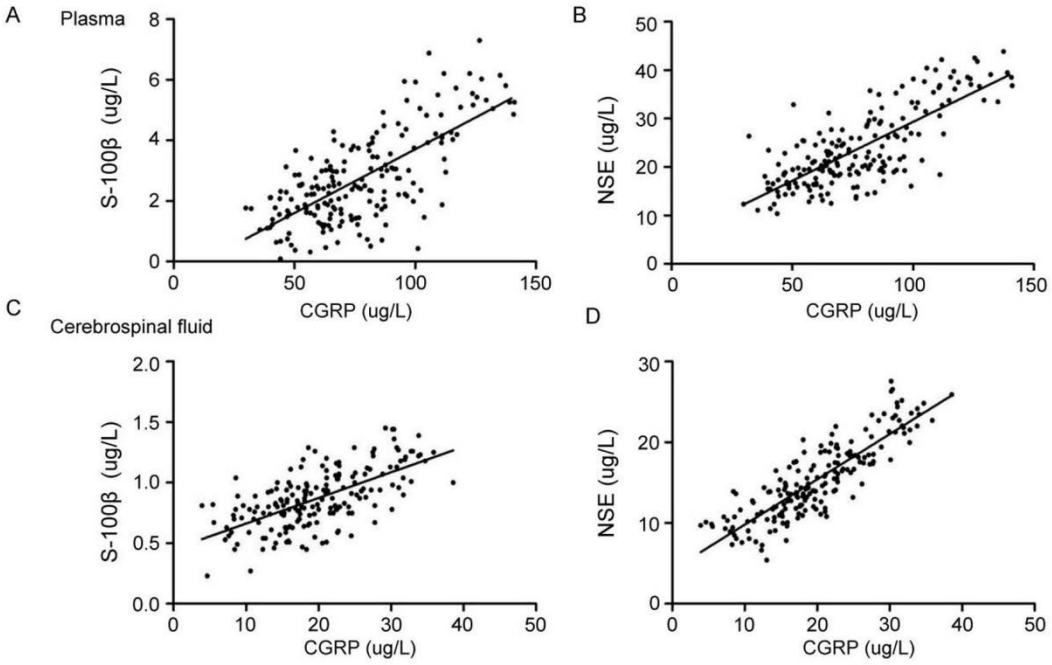

Figure 1. **Correlation of CGRP levels with S-100β and NSE in children with HIE.**

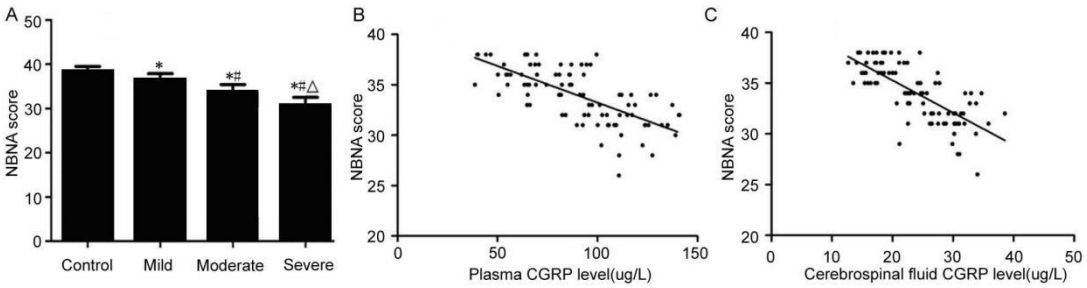

Figure 2. **NBNA score of each group and its correlation with plasma and cerebrospinal fluid CGRP levels. * Indicates comparison with the control group, P <0.05; #b indicates comparison with the mild group, P <0.05; △ indicates comparison with the moderate group, P <0.05.**