Original Article

The expression and clinical value of ubiquitin carboxyl-terminal hydrolase L1 in the blood of neonates with hypoxic ischemic encephalopathy

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Background: Neonatal hypoxic ischemic encephalopathy (HIE) can result in mental retardation due to the associated brain damage. Early identification of brain injury is vital for the prevention and treatment of brain damage in neonates. This study investigated the expression levels of serum ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) in neonates with HIE and its correlation with brain damage.

Methods: From January 2019 to December 2020, 56 cases of neonatal patients with HIE were selected as the observation group, and 60 cases of healthy newborns delivered in our hospital during the same period were selected as the control group. Blood samples were obtained from neonates and the serum expression of UCH-L1 was detected by enzyme-linked immunosorbent assays (ELISAs). The relationship between UCH-L1 and neonatal prognosis and clinical features was analyzed.

Results: Compared with the healthy control group, the serum levels of UCH-L1 in the observation group was significantly higher (2.28±1.21 *vs.* 0.81±0.39 ng/mL, P=0.000). Furthermore, at 6 hours after birth, the serum levels of UCH-L1 were significantly higher in neonates with moderate to severe HIE compared to patients with mild HIE (2.92±0.80 and 1.76±0.72 ng/mL, respectively, P=0.000). Pearson correlation analysis showed that the expression levels of UCH-L1 were negatively correlated with the development quotient (DQ), intelligence index (MI), and the Neonatal Behavioral Neurological Assessment (NBNA) score of HIE newborns (P<0.05).

Conclusions: The level of UCH-L1 protein expression is elevated in the serum of newborns with HIE, and this may have a certain clinical value in predicting the intelligence of children.

Keywords: Ubiquitin carboxy-terminal hydrolase L1 (UCH-L1); hypoxic ischemic encephalopathy (HIE); newborn; clinical value

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Introduction

Neonatal hypoxic ischemic encephalopathy (HIE) is one of the most common diseases in pediatrics. HIE has a complicated pathogenesis and is usually triggered by intrauterine distress or neonatal asphyxia during the perinatal period. Clinical signs of HIE includes drowsiness, irritability, and coma. Surviving neonates with HIE are often characterized by delayed development, cerebral palsy, and even death (1-3). Therefore, biomarkers that can detect early intracranial injury is crucial. In the last decade, research has shown that acute brain injury is characterized by the presence of ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) (4,5). In neonates with HIE, early stage ischemia and hypoxia result in different degrees of acute

Table 1 A comparison of the general patient characteristics

Variable	Observation group (n=56)	Control group (n=60)	t/χ^2 value	P value
Gender (male/female)	26/30	32/28	0.552	0.457
Gestational age (weeks)	38.51±1.45	38.47±1.13	0.166	0.868
Head circumference (cm)	33.65±3.46	34.53±3.74	1.313	0.192
Weight (kg)	3.25±0.43	3.32±0.45	0.855	0.394

brain injury. Thus, UCH-L1 may be a promising biomarker of brain damage in neonates with HIE and may be a useful predictor of later neurodevelopmental outcomes. This report investigated the significance of UCH-L1 in the early diagnosis of HIE. We present the following article in accordance with the MDAR reporting checklist (available at https://dx.doi.org/10.21037/tp-21-327).

Methods

Patients

A total of 56 neonates with HIE treated in our hospital from January 2019 to December 2020 were enrolled in this study as the observation group. Neonates were included in this study if they were delivered in our hospital and met the diagnostic criteria for neonatal HIE. Patients with infectious diseases of the nervous system, congenital metabolic diseases, or cerebral infarction were excluded. A total of 60 healthy newborns delivered in our hospital during the same period were randomly selected for the control group. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by institutional ethics board of the Ganzhou People's Hospital (No.: 2019-142) and informed consent was taken from all the patients.

Out of the 56 neonates in the observation group, there were 26 males and 30 females. The average gestational age was 38.51±1.45 weeks, and the average age was 3.25±2.73 days. The average head circumference was 33.65±3.46 cm, and the average weight was 3.25±0.43 kg. There were 31 neonates with mild HIE and 25 patients with moderate or severe HIE. The control group consisted of 32 males and 28 females. The average gestational age was 38.47±1.13 weeks. The average head circumference was 34.53±3.74 cm, and the average weight was 3.32±0.45 kg. There were no statistical significances in gender, gestational age, head circumference, weight, age, or any other general data between the two groups (P>0.05; *Table 1*).

Observation indicators and detection methods

To detect UCH-L1 expression, peripheral venous blood samples (2 mL) were obtained from all subjects at 6 hours after birth. After centrifugation at 3,000 rpm for 15 minutes, the supernatant was retained and the level of UCH-L1 expression in the serum was detected by enzyme-linked immunosorbent assays (ELISAs). The UCH-L1 ELISA kit was purchased from Ray Biotech Inc. (Ray Biotech, Atlanta Science Park, USA) and performed strictly according to the manufacturer's instructions.

To examine the clinical prognosis of the patients, the neuropsychological development of neonates in the observation group was evaluated by the development quotient (DQ) and the intelligence index (MI) according to the Developmental Screening Test. In addition, the Neonatal Behavioral Neurological Assessment (NBNA) score was used to evaluate the neurobehavioral development of newborns. The time points for assessment were 3, 14, and 28 days after birth.

Statistical analysis

All statistical analyses were performed using SPSS 26.0 software (IBM, Chicago, USA). A two-tailed P value <0.05 was considered statistically significant. Enumeration data were analyzed by the χ^2 test. Measurement data are presented as mean ± standard deviation (xxa). Comparison between two groups was performed using the *t*-test, while comparison of multiple groups was conducted using the F test. The correlation between two measurement data was analyzed using the Pearson correlation analysis.

Results

A comparison of serum UCH-L1 levels in the two groups

At 6 hours after birth, the serum levels of UCH-L1 in

Table 2 Serum levels of ubiquitin carboxy-terminal hydrolase L1 (UCH-L1)

	Observation group (n=56)	Control group (n=60)	t value	P value
UCH-L1 (ng/mL)	2.28±1.21	0.81±0.39	8.929	0

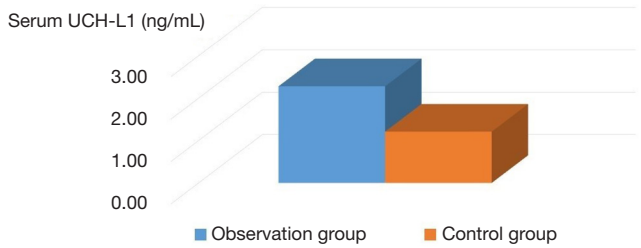

Figure 1 Serum levels of ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) in neonates.

Table 3 Serum levels of ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) in children with different degrees of hypoxic ischemic encephalopathy (HIE)

	Mild HIE (n=31)	Moderate to severe HIE (n=25)	t value	P value
UCH-L1 (ng/mL)	1.76±0.72	2.92±0.80	5.704	0

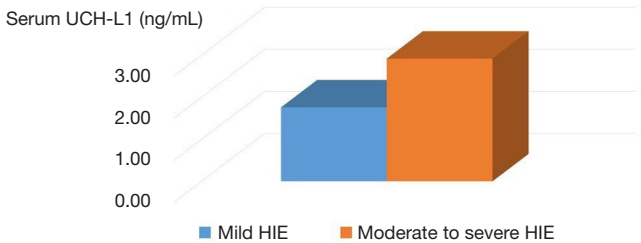

Figure 2 Serum levels of ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) in neonates with different degrees of hypoxic ischemic encephalopathy (HIE).

neonates with HIE was significantly higher than the levels observed in healthy neonates (2.28±1.21 and 0.81±0.39 ng/mL, respectively; P=0.000; *Table 2*; *Figure 1*).

A comparison of serum levels of UCH-L1 in children with different degrees of HIE

At 6 hours after birth, the levels of serum UCH-L1 in children with moderate to severe HIE was significantly higher than the levels observed in neonates with mild HIE (2.92±0.80 and 1.76±0.72 ng/mL, respectively, P=0.000; *Table 3*; *Figure 2*).

Clinical prognosis of neonates at follow-up

At the 6 months follow-up, the Developmental Screening Tests were performed on the neonates. The DQ, MI, and NBNA scores at different time points in the moderate to severe HIE group were significantly lower than those in the mild HIE group and the control group (P<0.05). The DQ, MI, and NBNA scores in the mild HIE group were also significantly lower compared to the control group

Table 4 Clinical prognosis of neonates at the 6 months follow-up

Variable	Control group (n=60)	Mild HIE (n=31)	Moderate to severe HIE (n=25)	F value	P value
DQ	92.68±5.62	88.52±5.53[a]	72.33±5.72[ab]	9.765	0
MI	93.24±3.79	88.55±5.03[a]	74.24±5.33[ab]	10.024	0
NBNA score					
3 d	37.98±0.82	37.32±0.76[a]	36.32±0.80[ab]	8.949	0
14 d	38.34±0.65	37.91±0.58[a]	37.34±0.52[ab]	9.124	0
28 d	38.82±0.62	38.03±0.69[a]	37.54±0.59[ab]	9.652	0

[a], P<0.05, compared with the control group; [b], P<0.05, compared with the mild HIE group. HIE, hypoxic ischemic encephalopathy; DQ, development quotient; MI, intelligence index; NBNA, Neonatal Behavioral Neurological Assessment.

Table 5 The correlation between ubiquitin carboxy-terminal hydrolase L1 expression and development quotient and intelligence index neonates with hypoxic ischemic encephalopathy

Variable	r value	P value
DQ	–0.452	0
MI	–0.446	0.001
NBNA score		
3 d	–0.413	0.002
14 d	–0.462	0
28 d	–0.398	0.01

DQ, development quotient; MI, intelligence index; NBNA, Neonatal Behavioral Neurological Assessment.

(P<0.05; *Table 4*).

The correlation between UCH-L1 expression and the development quotient and intelligence index in neonates with HIE

Pearson correlation analysis showed that UCH-L1 expression was negatively correlated with DQ, MI, and NBNA scores in HIE neonates (P<0.05; *Table 5*).

Discussion

Brain injury associated with neonatal HIE can be severe, and its sequelae include mental retardation, developmental retardation, and epilepsy (6). Approximately 0.3% of newborns with HIE develop cerebral ischemia and hypoxic injury, and about 15% of children with HIE present with varying degrees of sequelae (7,8). At present, the diagnosis of HIE relies heavily on computed tomography (CT), magnetic resonance imaging (MRI), and other imaging modalities, which are relatively subjective. Therefore, the development of potential serum biomarkers is vital for the diagnosis of HIE. UCH-L1 hydrolyzes the chain between ubiquitin and substrate protein, and then the substrate enters the proteasome and is degraded. UCH-L1 is a protein distinctly expressed in brain cells, and its levels are related to the degree of neuronal brain damage and the destruction of the blood-brain barrier, which is indicative of nervous system injury (9-11).

This study demonstrated that serum levels of UCH-L1 were significantly elevated in children with HIE (the observation group) compared with neonates in the healthy control group at 6 hours after birth. Furthermore, the levels of UCH-L1 in neonates with moderate to severe HIE were significantly higher than that in neonates with mild HIE at 6 hours after birth. These results are consistent with earlier studies (12,13). Previous work in rat models have shown that the concentration of UCH-L1 increases in the presence of brain damage (14), and the elevation in UCH-L1 levels reaches a peak at 6 hours after birth in brain-damaged human neonates (15). During HIE, brain injury results in the release of inflammatory factors, excessive production of free radicals, and other related phenomena, which interact with each other, leading to degeneration, necrosis, and apoptosis of brain neurons and the destruction of the blood-brain barrier (16-18). At this time, the brain releases a large amount of UCH-L1 protein which enters the blood through the damaged blood-brain barrier, and can thus be detected in the cerebrospinal fluid or blood during the acute stage of brain injury (19,20).

This study also revealed that the serum levels of UCH-L1 in neonates with moderate to severe HIE was significantly higher than that in neonates with mild HIE

(P=0.000) at 6 hours after birth. Pearson correlation analysis showed that the levels of UCH-L1 were significantly negatively correlated with DQ, MI, and NBNA score (P<0.05). The results indicated that the levels of UCH-L1 increase with the progression of the disease, and the higher its concentration, the poorer the prognosis.

In conclusion, the expression levels of UCH-L1 protein can be used for the diagnosis of acute brain injury caused by HIE, and can reflect the severity of HIE and predict the prognosis of children with HIE. Therefore, serum levels of UCH-L1 may be a potential biomarker in clinical practice. We need further studies to investigate whether inhibition of UCH-L1 expression could attenuate brain injury in neonates with HIE or not.

Limitations

Limited to ethical requirements, we could not detect the expression of UCH-L1 in the brain.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://dx.doi.org/10.21037/tp-21-327). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by institutional ethics board of the Ganzhou People's Hospital (No.: 2019-142) and informed consent was taken from all the patients.

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