Clin. Lab. 2017;63:XXX-XXX

ORIGINAL ARTICLE

Predictive Value of Neurodevelopmental Outcome and Serum Tau Protein Level in Neonates with Hypoxic Ischemic Encephalopathy

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SUMMARY

Background: **Tau protein is s specific protein expressed by neurons in the central nervous system. Elevated serum Tau protein is associated with many diseases of the central nervous system. The serum Tau protein level in neonates with hypoxic ischemic encephalopathy (HIE) is still poorly understood.**
Methods: **Forty-one human neonates with HIE and thirty-five healthy neonates (control group) within 24 hours after birth were studied. Tau protein in serum was detected by enzyme-linked immunosorbent assay. Neurological outcome was assessed at 9 months of age according to the Gesell developmental scale.**
Results: **Tau protein in serum was significantly higher in the HIE group than in the control group ($p < 0.01$), in neonates with severe HIE than neonates with moderate HIE ($p < 0.01$), and in infants with neurodevelopmental retardation compared with those with normal neurodevelopment ($p < 0.01$). The result of this study showed an obvious negative correlation between the serum Tau protein level and development quotients of neonates with HIE ($r_s = -0.6172$, $p < 0.01$). Receiver operator characteristic curve analysis showed that Tau protein (cutoff value 933.04 pg/mL) was a predictor of neurodevelopmental retardation outcome (AUC value = 0.860 (95% CI: 0.736 - 0.983, $p < 0.01$), sensitivity 100%, specificity 70.8%).**
Conclusions: **Serum Tau protein level within 24 hours after birth can be used as a marker for the early diagnosis of neonatal HIE and predicting neurodevelopmental outcomes.**
(Clin. Lab. 2017;63:xx-xx. DOI: 10.7754/Clin.Lab.2017.170103)

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Manuscript accepted February 6, 2017

KEY WORDS

neonate, hypoxic ischemic encephalopathy, Tau protein, development quotient, neurodevelopmental prognosis

INTRODUCTION

Moderate/severe neonatal hypoxic ischemic encephalopathy (HIE) is a major cause of death, neurodevelopmental disorders, and neurological disability in full-term neonates. It is estimated that about five million newborns die each year worldwide, and about 19% of

the deaths are caused by asphyxia at birth. Asphyxia in the term of delivery was 0.5% [1] and its prognosis depends on the severity of asphyxia, neonates with mild asphyxia have a good prognosis, whereas, neonates with severe asphyxia often have associated multiple organ injury, especially brain injury, with a bad prognosis. In developed countries, neonatal HIE occurs in 1.5/1000 full-term infants and in almost 60% of premature neonates [2]. Approximately 20% of affected neonates die, and 25 - 30% of the patients will develop a permanent neurodevelopmental disorder or cognitive dysfunction [3-9]. In China, the incidence of neonatal asphyxia is 3.78% (severe asphyxia for about 0.75%) [10]. Therefore, it is very important to screen and determine the functional state of the brain in neonatal asphyxia as early as possible to develop an intervention plan. During the neonatal asphyxia-resuscitation period, the symptoms may be hidden by unconsciousness or the application of anticonvulsant drugs. The diagnosis, grading, and prognosis of neonatal HIE relies on Apgar score, umbilical artery blood pH, Sarnat score, cranial tomography (CT), magnetic resonance imaging (MRI), ultrasonic diagnosis, and electroencephalogram (EEG) monitoring methods [11-18]. However, because the development and evolution of the disease is very complex, which can be influenced by other factors, there are some limitations to the types of examination and assessment methods that can be used. Many clinicians test brain specific proteins or other biological markers in the blood or cerebrospinal fluid after the newborn asphyxia insult. The measurement of brain injury biomarkers for the early diagnosis and neurodevelopment prediction in neonatal HIE is critical to understand quickly and accurately whether the brain is damaged and to provide intervention measures early. This has been gaining increased attention in recent years. Tau protein is a specific protein expressed by central neurons, and its level in the cerebrospinal fluid or blood reflects the degree of injury of neurons. There have been more studies of Tau protein in adults with brain damage compared with neonatal brain damage, and the use of Tau protein for the diagnosis and prognosis of neonatal HIE is still poorly understood. Therefore, it is necessary to study the prognostic value of Tau protein in neonatal HIE. In this study, serum Tau protein levels of 41 neonates with moderate/severe HIE were studied by enzyme-linked immunosorbent assay to determine whether serum Tau protein level is useful for the early diagnosis, clinical grading, and neurodevelopmental outcome of neonatal HIE.

MATERIALS AND METHODS

Study population

Forty-one neonates with moderate/severe HIE admitted (August 2014 through August 2015) to the Neonatal Intensive Care Units (NICU) of The Handan Maternal and Child Health Care Hospital of Hebei province were enrolled in the study (HIE group). At the same time, 35 healthy term neonates in our hospital were randomly selected as the control group. This study was conducted in accordance with the Declaration of Helsinki and was approved by the Medical Ethics Committee of Handan Maternal and Child Health-Care Hospital of Hebei province. Written informed consent was provided by the legal guardians of all participants.

HIE population

Entry criteria of forty-one neonates with moderate/severe HIE [19]: (1) neonates with a gestational age ≥ 37 weeks, weight ≥ 2500 g; (2) severe asphyxia at birth, Apgar score of ≤ 3 at 1 minute or Apgar score of ≤ 5 at 5 minutes; (3) umbilical artery blood pH ≤ 7.0 at birth; and (4) abnormal neurological signs present in the first 24 hours of life (drowsiness or coma, convulsions, abnormal muscle tone, the original reflex to weaken or disappear, irregular breathing, pupil size and light reflection anomaly), or neonates with HIE with abnormal EEG, and brain abnormalities confirmed by CT scan or MRI. Exclusion criteria: (1) severe intracranial hemorrhage; (2) congenital malformations or inherited metabolic disease; (3) severe infectious disease; (4) mother with a history of drug abuse; (5) severe anemia, hemoglobin < 120 g/L; (6) neonates with automatic waiver treatment and incomplete follow-up. All neonates with HIE were admitted to NICU received respiratory support, convulsion control, and had reduced intracranial pressure, maintenance of blood perfusion to brain and body, maintenance of blood sugar at a normal high value, hypothermia or nerve protective drugs and other comprehensive treatments. Tests of blood, liver function, renal function, blood glucose, and blood pressure were also performed for all patients after admission.

Control population

Thirty-five full-term, healthy neonates fulfilled all of the following criteria: mother's health, no asphyxia at birth, respiratory distress symptoms did not occur within the first 3 days of postnatal life, and umbilical artery blood pH ≥ 7.2 in the first 24 hours of life. Exclusion criteria: Congenital dysplasia, congenital metabolic disease, severe infection, mother with high blood pressure, diabetes, and drug use during pregnancy.

Measurement of serum Tau protein

Blood sample was taken from the vein within the first 24 hours of postnatal life, and were placed in test tubes (5 mL, No. A06277602, Hebei City Zhong Xing Medical Supplies Co. Ltd, China) and kept for 30 minutes at room temperature, centrifuged (table low speed automatic balancing centrifuge L-400; Hunan Xiangyi Laboratory Instrument Development Co. Ltd., China) for 15 minutes (3000 r/minute). The serum was loaded into a test tube and kept at -70°C until assay. To avoid an influence on the detection results, the neonates did not have a blood transfusion before collecting blood samples. Serum Tau protein level was detected by enzyme-

linked immunosorbent assay kits (Shanghai HuDing Biological Science and Technology Co., Ltd. [R&D Systems, USA]; Tau protein detection range: 30 - 1200 pg/mL). Before the serum was detected, the samples were removed from the -70°C freezer into the room temperature, and sample was placed on an oscillator (FWZ-1 type micro drug device, Guangzhou Fenghua Bioengineering Co. Ltd, China), with shock melting for 1 hour (1200 r/minute). The assay was performed following the manufacturer's instructions.

Clinical grading of neonatal HIE

The clinical grading of neonatal HIE was according to the criteria of Sarnat and Sarnat [20]. HIE was defined as "mild" if hyperexcitability or hypotonia persisted without seizures for at least 72 hours after birth; "moderate" if the infant was lethargic and had hypotonia, with weak primitive reflexes and seizures; and "severe" if the infant suffered frequent seizures, apnea, flaccid weakness or coma.

Neurodevelopment outcome assessment

In this study, the neurodevelopment outcome of neonates with HIE were assessed at 9 months of age, then by follow-up by hospital referral or home visit. The Gesell development scale was used as an evaluation tool to assess neurodevelopment: it assesses adaptability, gross motor, fine motor, language, personal social and 5 other areas, and the development quotient (DQ) [21,22]. Evaluation criteria: Neurodevelopment retardation was determined if the parameters of DQ were < 75 score (1 or more than 1 area), boundary situation was defined if the parameters of DQ had a score of 75 - 85, and good neurodevelopment was defined if the DQ score was > 85. For this study, neurodevelopment prognosis was evaluated by experienced pediatric rehabilitation physicians.

Statistical analysis

Dates were analyzed using SPSS software version 11.5 (SPSS, Chicago, IL, USA). Measurement dates are expressed as the mean ± standard deviation; the variances of the serum Tau protein level in the controls and HIE group were analyzed using Student's *t*-test; the comparison of multiple groups were calculated using analysis by one way ANOVA. Enumeration dates are expressed using the number and percentage, and the comparison between groups used the Chi-square test. The correlation between serum Tau protein level and development quotient was tested by Spearman's rank-order correlation analysis. The specificity and sensitivity between the serum Tau protein level and the neurodevelopment prognosis were tested by receiver-operating characteristic (ROC) curves to evaluate the optimal cutoff values. In all statistical analyses, a p-value < 0.05 or < 0.01 was considered statistically significant.

RESULTS

The characteristics of the subjects and mothers are presented in Table 1. There were no statistically significant differences in gestational age, gender, weight, height, head circumference, mode of delivery, and mother parameters ($p > 0.05$) between the HIE and control groups. Of 41 neonates in the HIE group, there were 18 cases of abnormal umbilical cord, 8 cases of dystocia, 8 cases of abnormal placental or fetal membranes, 4 cases of neonatal respiratory distress syndrome, and 3 cases of unknown reasons. According to the criteria of Sarnat and Sarnat, neonates with HIE were classified into two groups: 15 cases of moderate HIE and 26 cases of severe HIE. According to the prognosis of neurodevelopment, neonates with HIE were divided into three groups: 11 cases of neurodevelopmental retardation, 24 cases of good neurodevelopment, and 6 cases of boundary situation.

Comparison of serum Tau protein level in HIE group with control group

Serum Tau protein level was detected in 41 neonates with HIE. Serum Tau protein level (884.88 ± 250.26 pg/mL; $p < 0.01$) was significantly higher in the HIE group than in the control group (106.41 ± 18.66 pg/mL).

Comparison of serum Tau protein levels in different groups of neonates

The results show that serum Tau protein level was significantly higher in the severe HIE group (999.63 ± 190.53 pg/mL; $p < 0.01$) than in the moderate HIE group (685.98 ± 217.41 pg/mL) and in the control group (106.41 ± 18.66 pg/mL). The serum Tau protein level was significantly higher in the moderate HIE group than in the control group ($p < 0.01$) (Figure 1).

Comparison of serum Tau protein levels with different neurodevelopmental outcomes of neonates with HIE

Serum Tau protein levels in neonates with neurodevelopmental retardation, good neurodevelopment, and boundary situation were further analyzed. Serum Tau protein level (1089.57 ± 73.61 pg/mL; $p < 0.01$) was significantly higher in neonates with neurodevelopmental retardation than in those with good neurodevelopment (755.18 ± 245.10 pg/mL), serum Tau protein level was not significantly higher in neonates with neurodevelopmental retardation than in those with boundary situation (1028.43 ± 127.29 pg/mL; $p > 0.05$); serum Tau protein level was significantly higher in neonates with boundary situation than in those with good neurodevelopment ($p < 0.01$) (Figure 2).

Correlation between serum Tau protein level and developmental quotients of neonates with HIE

The result show that the between serum Tau protein level and developmental quotient was obviously negatively correlated in neonates with HIE ($r_s = -0.6172$, p

Table 1. Important characteristics and clinical parameters of the mother and neonate on admission.

	HIE group (n = 41)	Control group (n = 35)	p-value
Maternal			
Mode of delivery, n (%)			
Cesarean	19 (45)	15 (43)	0.7607
Spontaneous vaginal delivery	18 (44)	20 (57)	0.2499
Perineotomy	4 (9)	0 (0)	0.1606
Age (years), mean ± SD	27.22 ± 5.62	27.76 ± 4.83	0.3720
Gravida			
Gestational hypertension, n (%)	3 (7)	0 (0)	0.2974
Diabetes mellitus, n (%)	1 (2)	0 (0)	1.0000
Uterine inertia, n (%)	3 (7)	0 (0)	0.2974
Clinical chorioamnionitis, n (%)	1 (2)	0 (0)	1.0000
Neonatal			
Admission age (years), hours (mean ± SD)	4.03 ± 6.84	3.87 ± 5.34	0.9110
Gestational age (years), weeks (mean ± SD)	39.23 ± 1.28	39.12 ± 1.15	0.0757
Gender			
Male, n (%)	25 (61)	21 (60)	0.3145
Female, n (%)	16 (39)	14 (40)	0.9309
Weight, g (mean ± SD)	3369.02 ± 514.43	3325.12 ± 491.28	0.4849
Length, cm (mean ± SD)	50.43 ± 2.65	50.65 ± 2.83	0.4989
Head circumference, cm (mean ± SD)	33.63 ± 1.32	33.02 ± 1.17	0.0902
Parity, n (%)			
First child	19 (46)	22 (63)	1.0000
Second child	12 (29)	13 (37) ▵	0.0136
Third child	7 (17)	0 (0) ▵	0.0302
Fourth child	1 (2)	0 (0)	1.0000
Sixth child	2 (5)	0 (0)	0.4965
Cause of HIE			
Abnormality of umbilical cord, n (%)			
Cord around neck	8 (20)	0 (0) ▵	0.0169
Cord around neck + RDS	2 (5)	0 (0)	0.4965
Cord around neck + Rupture of membranes	2 (5)	0 (0)	0.4965
Cord around neck + Placental abruption	1 (2)	0 (0)	1.0000
Cord around neck + Aging of placenta	1 (2)	0 (0)	1.0000
Cord around neck + Breech position	1 (2)	0 (0)	1.0000
Short cord	2 (5)	0 (0)	0.4965
Torsion of cord	1 (2)	0 (0)	1.0000
Dystocia, n (%)			
Malposition	3 (7)	0 (0)	0.2974
Uterine inertia	3 (7)	0 (0)	0.2974
Cephalopeluic disproportion	1 (2)	0 (0)	1.0000
Oligohydramnions	1 (2)	0 (0)	1.0000
Placenta and membrane abnormality, n (%)			
Placental abruption	1 (2)	0 (0)	1.0000
Placental abruption + RDS	1 (2)	0 (0)	1.0000
Aging of placenta + RDS	1 (2)	0 (0)	1.0000

Table 1. Important characteristics and clinical parameters of the mother and neonate on admission (continue).

Rupture of membranes	**4 (10)**	**0 (0)**	**0.1666**
Clinical chorioamnionitis	**1 (2)**	**0 (0)**	**1.0000**
RDS, n (%)	**4 (10)**	**0 (0)**	**0.1666**
Unexplained asphyxia, n (%)	**3 (7)**	**0 (0)**	**0.2974**
Amniotic fluid excrement n (%)			
I ^{0}	**4 (10)**	**0 (0)**	**0.1666**
II ^{0}	**5 (12)**	**0 (0)**	**0.0943**
III ^{0}	**12 (29)**	**0 (0) ▵**	**0.0005**
Apgar Score, min (mean ± SD)			
Apgar Score 1 minute	**2.10 ± 0.60**	**8.42 ± 1.05 ▵**	**0.0000**
Apgar Score 5 minutes	**3.51 ± 1.20**	**8.60 ± 1.03 ▵**	**0.0000**
Apgar Score 10 minutes	**5.70 ± 1.30**	**8.54 ± 1.02 ▵**	**0.0000**
Clinical grading of HIE, n (%)			
Moderate	**15 (54)**	**0 (0) ▵**	**0.0001**
Severe	**26 (63)**	**0 (0) ▵**	**0.0000**
Oxygen supply mode, n (%)			
Mechanical ventilation	**14 (34)**	**0 (0) ▵**	**0.0001**
CPAP	**8 (20)**	**0 (0) ▵**	**0.0169**
Oxygen supply by head cover	**19 (46)**	**0 (0) ▵**	**0.0000**
Umbilical arterial blood test			
PH, value (mean ± SD)	**6. 82 ± 0.13**	**7.26 ± 0.09 ▵**	**0.0000**

HIE - Hypoxic ischemic encephalopathy, SD - standard deviation, RDS - respiratory distress syndrome.
▵ p < 0.05 vs. control group.

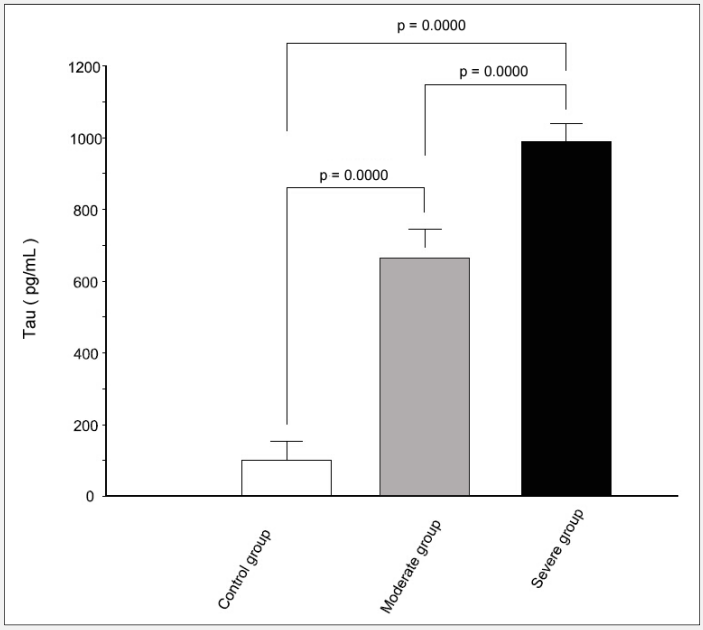

Figure 1. Comparison of serum Tau protein levels in different groups of neonates.

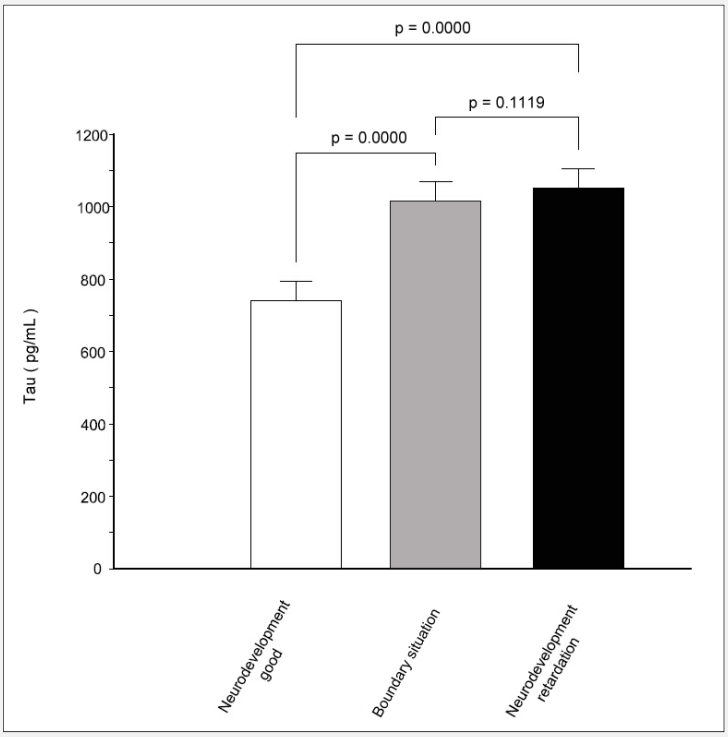

Figure 2. Comparison of serum Tau protein levels with different neurodevelopmental outcomes of neonates with HIE.

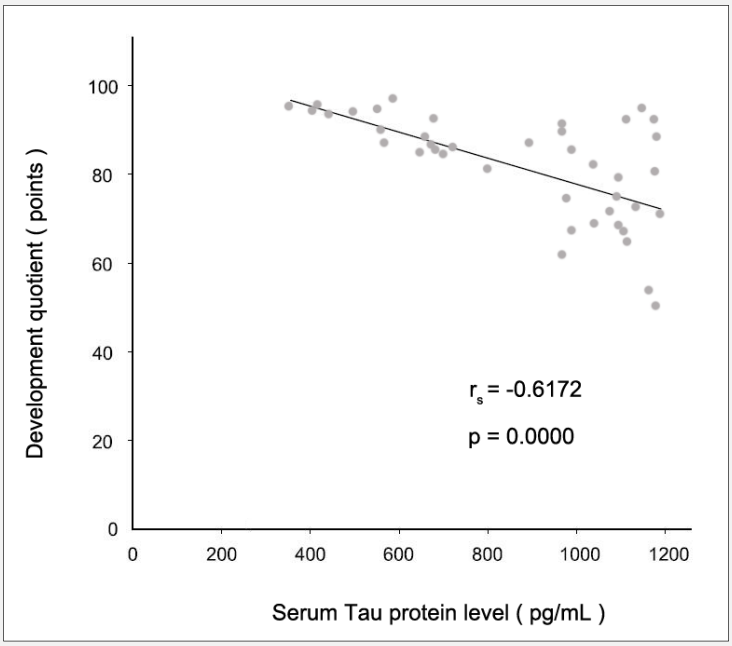

Figure 3. Correlation between serum Tau protein level and developmental quotients in neonates with HIE.

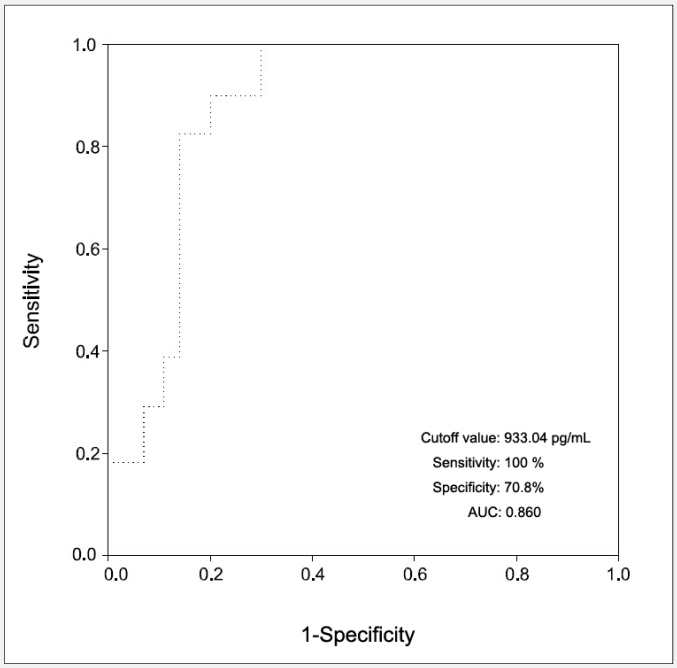

Figure 4. Receiver-operating characteristic curve of the serum Tau protein level with neurodevelopmental retardation prediction.

< 0.01), the higher the Tau protein level, the lower the score of developmental quotient (Figure 3).

Prediction of serum Tau protein level for neonates with neurodevelopmental retardation

In this study, to determine the prediction value of serum Tau protein level for neonates with neurodevelopmental retardation, the optimal critical value was obtained by drawing a ROC curve. The serum Tau protein level (933.04 pg/mL) was used as the cutoff value to obtain a more accurate prediction of the neurodevelopmental retardation at 9 months of age. The AUC value was 0.860 (95% CI: 0.736 - 0.983), with a sensitivity of 100%, specificity of 70.8%, and Youden index of 0.708 (Figure. 4).

Neurodevelopmental outcome at follow-up

The neurodevelopmental outcome of 41 patients was evaluated by Gesell Development schedules (GDS) at 9 months of age. The results shown include 11 cases (26.83%) of neurodevelopmental retardation (including 10 cases of severe HIE and 1 case of moderate HIE), 6 cases (14.63%) of boundary situation (including 4 cases of severe HIE and 2 cases of moderate HIE), and 24 cases (58.53%) of good neurodevelopment (including 12 cases of severe HIE and 12 cases of moderate HIE). According to the data analysis, the incidence rate of neurodevelopmental retardation was significantly higher ($X^2 = 11.5294$, $p < 0.05$) in neonates with severe HIE (incidence rate = 90.9%) compared with neonates with moderate HIE (incidence rate = 9.09%).

DISCUSSION

Neonatal HIE is a disease with high mortality that causes neurological disability. How to diagnose neonatal HIE early, provide early intervention, and reduce the incidence and disability rate, has become a hot spot for perinatal medicine workers.

Tau protein was identified as a microtubule-assembly factor in 1975. Hyperphosphorylated, insoluble, filamentous tau was shown to be the main component of neurofibrillary tangles (NFTs). Tau can be subdivided into four regions: an N-terminal projection region, a proline-rich domain, a microtubule-binding domain (MBD), and a C-terminal region [23]. Under normal conditions, Tau protein has a certain degree of phosphorylation, Tau phosphorylation can promote its binding to microtubules, is associated with microtubule assembly or stability, and is an essential factor in the development of axon growth and the formation of neuronal polarity. The increased expression of Tau protein after hypoxic/ischemic brain damage may be caused by

the interaction of calmodulin kinase, glycogen synthase kinase 3β (GSK-3β), and excitatory amino acid glutamate receptors. The hyperphosphorylation of Tau may result in the loss of tubulin polymerization, increased microtubule solubility, decreased cytoskeleton stability, decreased axon elongation, and impaired nutrient transport; all these changes will eventually lead to cell death [24,25].

Tau protein, a scaffold protein of nerve cells that belongs to the family of microtubule-associated proteins, is mainly distributed in the central nervous system in neuronal cell bodies and axons. Under normal circumstances, Tau protein maintains the stability and activity of microtubules in neurons, regulates the growth and development of neurons, participates in axon growth, and maintains the polarity formation of neurons and axonal communication [26]. After the neuron injury (included in hypoxia, trauma, etc.), Tau protein drops from microtubules, free Tau protein is then released from CNS neurons into the extracellular space and through the cerebrospinal fluid into the blood; therefore, the level of serum Tau protein can directly reflect the severity of neuronal damage [27,28]. Regarding the distribution of Tau protein in brain tissue, immunohistochemistry showed that Tau expression was high in the neocortex, hippocampus, and cerebellum, but segregated into the axon-neuropil domain of neurons in human brain tissue [29]. The first study in which Tau protein could be successfully analyzed in cerebrospinal fluid (CSF) by enzyme-linked immunosorbent assay was published in 1995 and showed that the Tau protein concentration was significantly elevated in Alzheimer's (AD) patients with control as well as in patients with neurodegenerative disorders [30,31]. Subsequently, many scholars further confirmed that the Tau protein in cerebrospinal fluid of the Alzheimer disease patients has been shown significantly increased with a sensitivity and specificity of 89 - 90% [32,33]. At present, enzyme-linked immunosorbent assay (ELISA) has been used extensively to determine cerebrospinal fluid or blood Tau protein concentration for brain injury diseases in clinical practice, such as, cerebral ischemia, brain trauma, brain hemorrhage, and large artery surgery lead to brain injury. Bielewicz J, et al. investigated the serum levels of Tau protein in 56 patients with cerebral ischemia. Tau protein was detected in the serum of 47.8% patients with cerebral ischemia, and the patients were found to have a large volume of cerebral infarction by cranial computed tomography scan and developed more severe neurological disability [34]. Ost M, et al. studied the levels of Tau protein in cerebrospinal fluid detected in patients with traumatic brain injury and higher levels of Tau protein were found. The level Tau protein of > 2,126 pg/mL on day 2 to 3 discriminated between dead and alive (sensitivity of 100% and specificity of 81%). A level of Tau protein > 702 pg/mL on day 2 to 3 discriminated between bad and good outcome (sensitivity of 83% and specificity of 69%) [35]. Hu TH, et al. studied the serum levels of Tau protein in 176 patients with cerebral hemorrhage; 110 cases (65.5%) had a poor outcome at 3 months, and mortality rate was 36% (64/176) at 3 months [36]. It has also been shown that Tau protein level was elevated in the ischemia stroke [37]. It is generally believed that Tau protein is a sensitive marker of neuronal degeneration or neuronal/axonal injury, and its level can reflect the severity of brain injury. In recent years, the enzyme-linked immunosorbent assay has been used in the detection of neonatal diseases. In order to study the changes of serum Tau protein level in neonatal jaundice encephalopathy, Okumus et al. studied the serum Tau and S100B protein levels by an enzyme-linked immunosorbent assay. The results show that serum Tau protein levels of neonates who manifested auditory neuropathy, neurologic abnormalities, or electroencephalogram abnormalities were significantly higher than in neonates without these abnormalities, and predicted a cutoff level of 265 pg/ mL. They thought that the serum Tau protein level in jaundiced term neonates were strongly correlated with early-phase bilirubin encephalopathy [38]. To study the relationship of neonatal asphyxia and serum Tau protein levels, Takahashi et al. studied 19 cases of neonatal asphyxia and found that the serum levels of Tau by an enzyme-linked immunosorbent assay were significantly higher in the neonates with a poor prognosis than in those with a good prognosis [39]. Tunc T. et al. studied the relationship between delivery type and Tau protein level in cord blood by an enzyme linked immunosorbent assay, the results show that serum Tau protein levels in the caesarean section group (79 pg/mL) were significantly lower than normal vaginal delivery (135 pg/mL) and forceps application group (175 pg/mL). They thought that caesarean section in this manner might be considered especially in conditions of risk of perinatal asphyxia to avoid hypoxia [40]. Based on the above research, it is feasible to detect neonatal brain damage disease testing Tau protein level by an enzyme linked immunosorbent assay. Up to now, serum Tau protein level in neonates with HIE is still poorly understood. To investigate the relationship between Tau protein and neonatal HIE, we detected serum Tau protein levels of neonates with HIE and 35 healthy full term neonates. The results showed that serum Tau protein levels in the HIE group were significantly higher than in the control group ($p < 0.05$). Our study also showed that the serum Tau protein level was significantly higher in the severe HIE group than in the moderate HIE group, and an obvious negative correlation between the serum Tau protein and developmental quotients, indicating that the higher the serum Tau protein level, the more serious the HIE situation; the higher the serum Tau protein level, the lower the development quotient, and the worse the neurodevelopmental outcome. ROC analysis showed that serum Tau protein levels at 24 hours after birth (cutoff value 933.04 pg/ mL) was a predictor of the neurodevelopmental retardation outcome of neonatal HIE (AUC value: 0.860 (95% CI: 0.736 - 0.983, $p < 0.01$), sensitivity and specificity were 100% and 70.8%. Therefore, the serum Tau pro-

tein levels at 24 hours after birth can be used as a biomarker for the early diagnosis of neonatal HIE and prediction of the severity and neurodevelopment outcome of neonates with HIE at 9 months of age. Of note, in this study, for 6 infants with a boundary situation neurodevelopmental outcome, we cannot predict the development direction of these patients, therefore, when we analysed the developmental retardation infants by ROC, 6 infants were not included. We also gave rehabilitation training guidance for patients and paid close attention to the development outcome of these infants during follow-up; we hope for a good result in these infants.

GDS is a diagnostic tool for the evaluation of neurological motor damage and mental disorders and is a more comprehensive response to the maturity of a child's nervous movement and intelligence development, which can more objectively reflect a child's nervous movement and mental development state. Therefore, 41 patients were followed-up and evaluated by Gesell's neurodevelopment assessment. The results showed that infants with neurodevelopment retardation accounted for 26.83%, good neurodevelopment accounted for 58.53%, and boundary situation accounted for 14.63%. Unfortunately, we could not complete the testing of the serum Tau protein level at 9 months of age.

CONCLUSION

Neonatal HIE is a complication after severe asphyxia, and its early diagnosis and treatment can reduce the mortality and morbidity rate of neonates with HIE. However, the current research is at *status quo*, and the therapeutic window for neonatal HIE is very narrow, within 6 hours of the HIE insult; therefore, it has become the focus of perinatal medicine to identify specific proteins of brain damage as biomarkers, to achieve early diagnosis, early intervention, and improve the prognosis of neonates with HIE. Serum Tau protein level was correlated with neonatal HIE, and its early detection might be helpful for the early diagnosis and neurodevelopmental prediction of neonatal HIE.

Acknowledgement:
This study was supported by the Health and Family Planning Commission of Hebei (NO: 20150033). Science and Technology Bureau of Handan City, Hebei Province (No: 152810879-6).

Declaration of Interest:
The authors declare no conflicts of interests.

References:

1. Zupan Simunek V. [Birth asphyxia in term newborns: Diagnosis, prognosis, neuroprotection]. Arch Pediatr 2010;17:578-82 (PMID: 20236812).
2. Kurinczuk JJ, White-Koning M, Badawi N. Epidemiology of neonatal encephalopathy and hypoxic-ischaemic encephalopathy. Early Hum Dev 2010;86:329-38 (PMID: 20554402).
3. Nelson KB, Ellenberg JH. Obstetric complications as risk factors for cerebral palsy or seizure disorders. JAMA. 1984;251:1843-8 (PMID: 6700086).
4. Vannucci RC, Perlman JM. Interventions for perinatal hypoxic-ischemic encephalopathy. Pediatrics 1997;100:1004-14 (PMID: 9374573).
5. Buonocore G, Perrone S, Longini M, et al. Non protein bound iron as early predictive marker of neonatal brain damage. Brain. 2003;126:1224-30 (PMID: 12690060).
6. Miller SP, Ramaswamy V, Michelson D, et al. Patterns of brain injury in term neonatal encephalopathy. J Pediatr 2005;146:453-60 (PMID: 15812446).
7. Gieron-Korthals M, Colón J. Hypoxic-ischemic encephalophathy in infant: New challenges. Fetal Pediatr Pathol 2005;24:105-20 (PMID: 16243755).
8. Nikas I, Dermenttzoglou V, Theofanopoulou M, Theodoropoulos V. Parasagittal lesions and ulegyria in hypoxic-ischemic encephalopathy: neuroimaging findings and review of the pathogenesis. J Child Neurol 2008;23:51-8 (PMID: 18160553).
9. Lai MC, Yang SN. Perinatal hypoxic-ischemic encephalopathy. J Biomed Biotechnol. 2011;2011:609813 (PMID: 21197402).
10. Wang QH, Yang YJ, Wei KL, Yao YJ, Du LZ. Epidemiological survey on newborn at the obstetric departments in hospitals in mid-southern region of China in 2005. Transl pediatr 2013;2:21-6 (PMID: 26835280).
11. Nelson KB, Ellenberg JH. Apgar scores as predictors of chronic neurologic disabilility. Pediatrics 1981;68:36-44 (PMID: 7243507).
12. Nelson KB, Grether JK. Potentially asphyxiating conditions and spastic cerebral palsy in infants of normal birth weight. Am J Obstet Gynecol. 1998;179:507-13 (PMID: 9731861).
13. Barkovich AJ, Hajnal BL, Vigneron D, et al. Prediction of neuromotor outcome in perinatal asphyxia:evaluation of MR scoring systems. AJNR Am J Neuroradiol 1998;19:143-9 (PMID: 9432172).
14. Yeh P, Emary K, Impey L. The relationship between umbilical cord arterial PH and serious adverse neonatal outcome: Analysis of 51,519 consecutive validated samples. BJOG 2012;119:824-31 (PMID: 22571747).
15. Thormton C, Rousset CI, Kichev A, et al. Molecular mechanisms of neonatal brain injury. Neurol Res Int 2012;2012:506320 (PMID: 22363841).
16. Thoresen M, Hellström-Westas L, Liu X, de Vries LS. Effect of hypothermia on amplitude-integrated electroencephalogram in infants with asphyxia. Pediatrics 2010;126:e131-9 (PMID: 20566612).
17. Hellstrŏm-Westas L, Rosen L, Svenningsen NW. Predictive value of early continuous amplitude integrated EEG recordings on outcome after severe birth asphyxia in full term infants. Arch Dis Child Fetal Neonatal Ed 1995;72:F34-8 (PMID: 7743282).

18. Rutherford M, Pennock J, Schwieso J, Cowan F, Dubowitz L. Hypoxic-ischaemic encephalopathy: early and late magnetic resonance imaging findings in relation to outcome. Arch Dis Child Fetal Neonatal Ed 1996;75:F145-51 (PMID: 8976678).

19. Group of Neonatology; Chinese Pediatric Society; Chinese Medical Association. [Diagnostic criteria for neonatal hypoxic-ischemic encephalopathy]. Zhonghua Er Ke Za Zhi. 2005;43:584 (PMID: 16191267).

20. Sarnat HB, Sarnat MS. Neonatal encephalopathy following fetal distress: a clinical and electroencephalographic study. Arch Neurol 1976;33:696-5 (PMID: 987769).

21. Roe KV. Correlation between Gesell scores in infancy and performance on verbal and non-verbal tests in early childhood. Percept Mot Skills 1977;45:1131-4 (PMID: 604891).

22. Li J, Bo T, Chen TQ, et al. Neurobehavioral development in preterm infants: a retrospective study of 181 cases. Zhongguo Dang Dai Er Ke Za Zhi 2014;16:696-700 (PMID: 25008875).

23. Mandelkow EM, Schweers O, Drewes G, et al. Structure, microtubule interactions, and phosphorylation of tau protein. Ann NY Acad Sci 1996;777:96-6 (PMID: 8624133).

24. Sydow A, Van der Jeugd A, Zheng F, et al. Tau-induced defects in synaptic plasticity, learning and memory are riversible in transgenic mice after switching off the toxic Tau mutant. J Neurosci 2011;31:2511-25 (PMID: 21325519).

25. Sarnat H, Flores-Sarnat L, Crino P, Hader W, Bello-Esponosa L. Hemimegalencephaly: foetal tauopathy with mTor hyperactivation and neuronal lipidosis. Folia Neuropathol 2012;22:380-1 (PMID: 23319189).

26. Hampel H, Blennow K, Shaw LM, Hoessler YC, Zetterberg Henrik, Trojanowski JQ. Total and phosphorylated Tau protein as biological markers of Alzheimei's disease. Exp Gerontol 2010: 45:30-40 (PMID: 19853650).

27. Morris M, Maeda S, Voseel K, Mucke L. The many faces of tau. Neuron 2011;70:410-26 (PMID: 21555069).

28. Liliang PC, Liang CL, Lu K, et al. Relationship between injury severity and serum tau protein levels in traumatic brain injured rats. Resuscitation 2010;81:1205-8 (PMID: 20598429).

29. Trojanowski JQ, Schuck T, Schmidt ML, Lee VM. Distribution of tau protein in thenormal human central and peripheral nervous system. J Histochem Cytochem 1989;37:209-15 (PMID: 24920 45).

30. Arai H, Terajima M, Miura M, et al. Tau in cerebrospinal fluid: a potential diagnostic marker in Alzheimer's. Ann Neurol. 1995;38: 649-52 (PMID: 7574462).

31. Blennow K, Wallin A, Agren H, Spenger C, Siegfried J, Vanmechelen E. Tau protein in cerebrospinal fluid: a biochemical marker for axonal degeneration in Alzheimer disease? Mol Chem Neuropathol 1995;26:231-45 (PMID: 8748926).

32. Blennow K, Zetterberg H. Cerebrospinal fluid biomarkers for Alzheimer's disease. J Alzheimers Dis 2009;18:413-7 (PMID: 19661632).

33. Shaw LM, Vanderstichele H, Knapik-Czajka M, et al. Cerebrospinal fluid biomarker signature in Alzheimer's disease neuroimaging initiative subjects. Ann Neurol. 2009;65:403-13 (PMID: 19296504).

34. Bielewicz J, Kurzepa J, Czekajska-Chehab E, Stelmasiak Z, Bartosik-Psujek H. Dose serum Tau protein predict the outcome of patients with ischemic stroke. J Mol Neurosci 2011;43:241-5 (PMID: 20549384).

35. Ost M, Nylén K, Csajbok L, et al. Initial CSF total correlates with 1 year outcome in patients with traumatic brain injury. Neurology 2006;67:1600-4 (PMID: 17101890).

36. Hu HT, Xiao F, Yan YQ, Wen SQ, Zhang L. The prognostic value of serum tau in patients with intracerebral hemorrhage. Clin Biochem 2012;45:1320-4 (PMID: 22705449).

37. Song B, Ao Q, Wang Z, et al. Phosphorylation of tau protein over time in rats subjected to transient brain ischemia. Neural Regen Res. 2013;8:3173-82 (PMID: 25206638).

38. Okumus N, Turkyilmaz C, Onal EE, et al. Tau and S100B proteins as biochemical markers of bilirubin-induced neurotoxicity in term neonates. Pediatr Neurol 2008;39:245-52 (PMID: 188053 62).

39. Takahashi K, Hasegawa S, Maeba S, et al. Serum tau protein level serves as a predictive factor for neurological prognosis in neonatal asphyxia. Brain Dev 2014;36:670-5 (PMID: 24268747).

40. Tunc T, Karaoglu A, Cayci T, et al. The relation between delivery type and tau protein levels in cord blood. Pediatrics int 2010;52: 872-5 (PMID: 21166947).