

Umbilical cord blood troponin I, myoglobin and CK-MB in neonatal hypoxic ischemic encephalopathy and the clinical significance

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Abstract. Associations of serum S-100 β , cystatin C (Cys-C) and C-reactive protein (CRP) with umbilical cord blood troponin I (TnI), myoglobin (Mb) and creatine kinase-MB (CK-MB) in neonatal hypoxic ischemic encephalopathy (NHIE) and the clinical significance were explored. A total of 40 patients with NHIE treated in the Binzhou Medical University Hospital were selected as observation group, while another 40 healthy neonates in the same period were selected as control group. The related data of all subjects were collected, and the levels of serum S-100 β protein, CRP and Cys-C, and umbilical cord blood TnI, Mb and CK-MB were compared between the two groups. Associations of the neonatal behavioral neurological assessment (NBNA) score with the changes in serum S-100 β protein, CRP and Cys-C and umbilical cord blood TnI, Mb and CK-MB were analyzed. The univariate and multivariate logistic regression analyses were performed to determine the risk factors for NHIE. In observation group, the levels of serum S-100 β protein, CRP and Cys-C were significantly higher than those in control group, and the levels of umbilical cord blood TnI, Mb and CK-MB were also significantly higher than those in control group. The NBNA score was negatively correlated with the changes in serum S-100 β protein, CRP and Cys-C as well as the umbilical cord blood TnI, Mb and CK-MB. The levels of serum S-100 β protein, CRP and Cys-C, and umbilical cord blood TnI, Mb and CK-MB were related risk factors for NHIE. The increased levels of serum S-100 β protein, CRP and Cys-C, and umbilical cord blood TnI, Mb and CK-MB were independent risk factors for NHIE. In NHIE patients, the levels of serum S-100 β protein, CRP and Cys-C, and umbilical

cord blood TnI, Mb and CK-MB all significantly increased, and they have negative correlation with the nervous system function after onset.

Introduction

Neonatal hypoxic ischemic encephalopathy (NHIE) can be caused by various perinatal factors, which mainly leads to neonatal ischemia and hypoxia in the central nervous system, and decline or even suspension of cerebral blood flow, as well as secondary fetal or neonatal hypoxic injury in the central nervous system (1). NHIE is the most common complication of neonatal asphyxia, and also the major cause of mental retardation in children. Studies have shown that ~30% of NHIE patients suffer from disorders of mental development (2). The moderate-severe NHIE patients, although survived, have severe neurological sequelae, such as cerebral palsy, epilepsy and dysgnosis, causing serious psychological and economic burden on the families of the child patient and society (3).

Asphyxia is considered as the most direct cause of NHIE (4), during which central nervous system lesions are caused by the decline in the neonatal blood oxygen saturation mostly due to fetal-maternal circulation and gas exchange disorders (5). A study proved (6) that NHIE may be complicated with multiple organ dysfunctions after onset, and the organ dysfunction becomes more severe with the increasing severity of asphyxia, exerting a more serious negative impact on the long-term prognosis, and even affecting the quality of life of the child patients. Therefore, early detection, diagnosis and treatment of NHIE are of important significance for reducing the morbidity and mortality rates of the disease, which is also an important method to improve the neonatal birth quality in China. The present study explored the associations of serum S-100 β , cystatin C (Cys-C) and C-reactive protein (CRP) with umbilical cord blood troponin I (TnI), myoglobin (Mb) and creatine kinase-MB (CK-MB) in NHIE patients.

Patients and methods

General data. A total of 40 patients with NHIE treated in the Binzhou Medical University Hospital (Binzhou, China) from March 2017 to June 2019 were selected as observation

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group, while another 40 healthy neonates in the same period were selected as control group. All child patients met the diagnostic criteria for NHIE of the Society of Pediatrics, Chinese Medical Association in 2015, and they had high-risk factors for perinatal asphyxia. According to the neonatal birth mode, gestational weeks, Apgar scores immediately after birth and at 1 and 5 min after birth, the history of birth asphyxia, and diagnosis of neurological symptoms, the following subjects were excluded: Mothers complicated with severe systemic infection during pregnancy or taking immunosuppressive drugs and/or glucocorticoids during pregnancy, neonates definitely with congenital genetic diseases and/or deformity after birth, mothers complicated with severe respiratory system, digestive system or urinary system infection, or premature rupture of membranes for more than 24 h during enrollment, or mothers who used analgesic sedative drugs in the perinatal period or definitely had hemorrhagic shock. In observation group, there were 26 males and 14 females, the gestational age at birth was 32-42 weeks with an average of 37.6 ± 0.4 weeks, and the birth weight was 2,000-4,500 g with an average of $3,200.0 \pm 100.0$ g. In control group, there were 25 males and 15 females, the gestational age at birth was 32-42 weeks with an average of 37.7 ± 0.5 weeks, and the birth weight was 2,000-4,500 g with an average of $3,200.5 \pm 100.5$ g. The sex, gestational age at birth and birth weight had no statistically significant differences between the two groups ($P > 0.05$). This study was approved by the Ethics Committee of the Hospital Attached to the Binzhou Medical University. Signed informed consents were obtained from all the parents of the child participants before the study.

Methods. The related data of all subjects were collected, and the levels of serum S-100 β protein, CRP and Cys-C, and umbilical cord blood TnI, Mb and CK-MB were compared between the two groups. Moreover, the associations of the neonatal behavioral neurological assessment (NBNA) score with the changes in serum S-100 β protein, CRP and Cys-C and umbilical cord blood TnI, Mb and CK-MB were analyzed. The univariate and multivariate logistic regression analyses were performed to determine the risk factors for NHIE.

Evaluation criteria. The specimens of serum S-100 β protein [enzyme-linked immunosorbent assay (ELISA) (R&D Systems), $<0.5 \mu\text{g/l}$], CRP (ELISA, $<10 \text{ mg/l}$) and Cys-C (ELISA, $0.51\text{--}1.09 \text{ mg/l}$) were obtained from the venous blood of neonates. The specimens of TnI (ELISA, $0\text{--}0.034 \text{ ng/ml}$), Mb (ELISA, $0\text{--}121 \text{ ng/ml}$) and CK-MB (ELISA, $109\text{--}245 \text{ ng/ml}$) were obtained from the umbilical cord blood via puncture after the umbilical cord was cut at 1-2 min postnatally, and they were sent for detection within 15 min. The neurobehavioral ability of neonates was examined using the NBNA under a quiet, warm and semi-dark environment, and the neonates were breast-fed and helped to sleep at 1 h before assessment. The NBNA score includes the behavioral ability (6 major items), passive muscle tension (4 major items), active muscle tension (4 major items) and primary reflex (3 major items). The score of ≥ 37 points indicates normal ability, score of 35-36 points indicates the suspected brain injury, and score of <35 points indicates brain injury. The lower the score is, the more severe the brain injury will be.

Table I. Comparison of levels of serum S-100 β protein, CRP and Cys-C between the two groups (mean \pm SD).

	S-100 β ($\mu\text{g/l}$)	CRP (mg/l)	Cys-C (mg/l)
Observation group	1.3 ± 0.2	24.4 ± 1.6	2.1 ± 0.1
Control group	0.4 ± 0.1	7.4 ± 0.3	0.8 ± 0.1
t-value	25.456	66.047	58.138
P-value	<0.001	<0.001	<0.001

CRP, C-reactive protein; Cys-C, cystatin C.

Table II. Comparison of levels of umbilical cord blood TnI, Mb and CK-MB between the two groups (ng/ml, mean \pm SD).

	TnI	Mb	CK-MB
Observation group	0.057 ± 0.002	145.3 ± 3.5	287.5 ± 15.3
Control group	0.023 ± 0.001	102.3 ± 1.8	189.2 ± 9.8
t-value	96.167	69.099	34.217
P-value	<0.001	<0.001	<0.001

TnI, troponin I; Mb, myoglobin; CK-MB, creatine kinase-MB.

Statistical analysis. Statistical Product and Service Solutions (SPSS) 20.0 software (IBM Corp.) was used for statistical processing. Measurement data were expressed as mean \pm standard deviation (mean \pm SD). t-test was performed for the comparison of means between two groups, and χ^2 test for the comparison of rates between two groups. $P < 0.05$ indicates statistically significant difference.

Results

Comparison of the levels of serum S-100 β protein, CRP and Cys-C between the two groups. In observation group, the levels of serum S-100 β protein, CRP and Cys-C were significantly higher than those in control group ($P < 0.05$) (Table I).

Comparison of the levels of umbilical cord blood TnI, Mb and CK-MB between the two groups. In observation group, the levels of umbilical cord blood TnI, Mb and CK-MB were also obviously higher than those in control group ($P < 0.05$) (Table II).

Correlation analysis between NBNA score and changes in serum S-100 β protein, CRP and Cys-C. The NBNA score was negatively correlated with the changes in serum S-100 β protein, CRP and Cys-C ($P < 0.05$) (Table III and Figs. 1-3).

Correlation analysis between NBNA score and changes in umbilical cord blood TnI, Mb and CK-MB. The NBNA score was also negatively correlated with the changes in umbilical cord blood TnI, Mb and CK-MB ($P < 0.05$) (Table IV and Figs. 4-6).

Table III. Correlation analysis between NBNA score and changes in serum S-100 β protein, CRP and Cys-C.

	r-value	P-value
Serum S-100 β protein level	-0.8535	<0.001
Serum CRP level	-0.7371	<0.001
Serum Cys-C level	-0.8115	<0.001

NBNA, neonatal behavioral neurological assessment; CRP, C-reactive protein; Cys-C, cystatin C.

Table IV. Correlation analysis between NBNA score and changes in umbilical cord blood TnI, Mb and CK-MB.

	r-value	P-value
Umbilical cord blood TnI level	-0.6716	<0.001
Umbilical cord blood Mb level	-0.7967	<0.001
Umbilical cord blood CK-MB level	-0.9140	<0.001

NBNA, neonatal behavioral neurological assessment; TnI, troponin I; Mb, myoglobin; CK-MB, creatine kinase-MB.

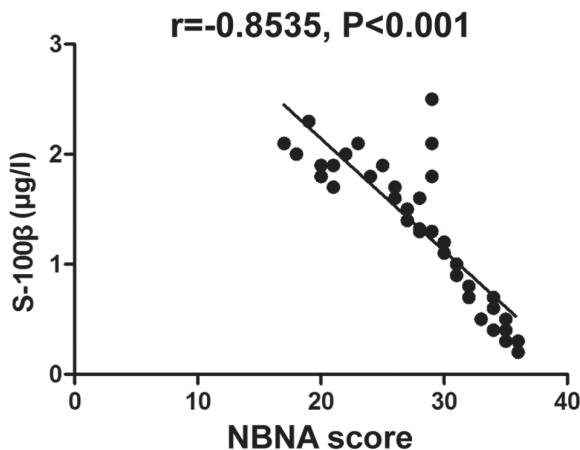


Figure 1. Correlation analysis between NBNA score and serum S-100 β protein level. NBNA, neonatal behavioral neurological assessment.

Univariate analysis for risk factors for NHIE. According to the univariate analysis, the levels of serum S-100 β protein, CRP and Cys-C, and umbilical cord blood TnI, Mb and CK-MB were related risk factors for NHIE (Table V).

Multivariate logistic regression analysis for risk factors for NHIE. According to the multivariate logistic regression analysis, the increased levels of serum S-100 β protein, CRP and Cys-C, and umbilical cord blood TnI, Mb and CK-MB were independent risk factors for NHIE (Table VI).

Discussion

The most important cause of NHIE is neonatal perinatal asphyxia, which results in neonatal hypoxic-ischemic injury

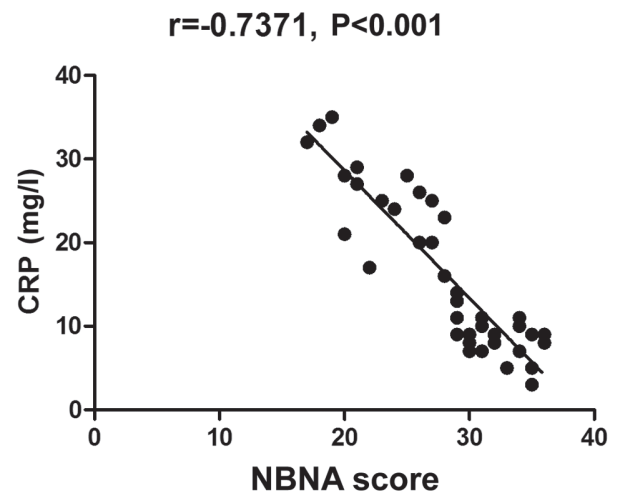


Figure 2. Correlation analysis between NBNA score and serum CRP level. NBNA, neonatal behavioral neurological assessment; CRP, C-reactive protein.

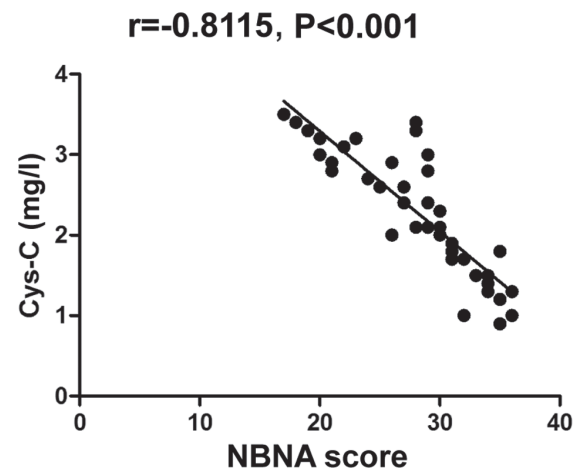


Figure 3. Correlation analysis between NBNA score and serum Cys-C level. NBNA, neonatal behavioral neurological assessment; Cys-C, cystatin C.

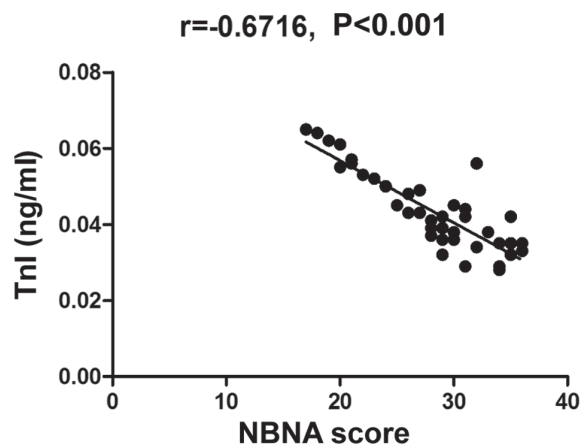


Figure 4. Correlation analysis between NBNA score and umbilical cord blood TnI level. NBNA, neonatal behavioral neurological assessment. NBNA, neonatal behavioral neurological assessment; TnI, troponin I.

in the central nervous system (7). In the case of delayed treatment, most child patients will die clinically (8), and there will

Table V. Univariate analysis for risk factors for NHIE (n).

	Onset	Normal	χ^2 value	P-value
Serum S-100 β protein level				
Rise	36	4	54.586	<0.001
Normal	2	38		
Serum CRP level				
Rise	35	5	55.000	<0.001
Normal	1	39		
Serum Cys-C level				
Rise	34	6	42.155	0.001
Normal	4	36		
Umbilical cord blood TnI level				
Rise	37	3	45.255	0.011
Normal	6	34		
Umbilical cord blood Mb level				
Rise	36	4	54.586	<0.001
Normal	2	38		
Umbilical cord blood CK-MB level				
Rise	38	2	57.836	<0.001
Normal	3	37		

NHIE, neonatal hypoxic ischemic encephalopathy; CRP, C-reactive protein; Cys-C, cystatin C; TnI, troponin I; Mb, myoglobin; CK-MB, creatine kinase-MB.

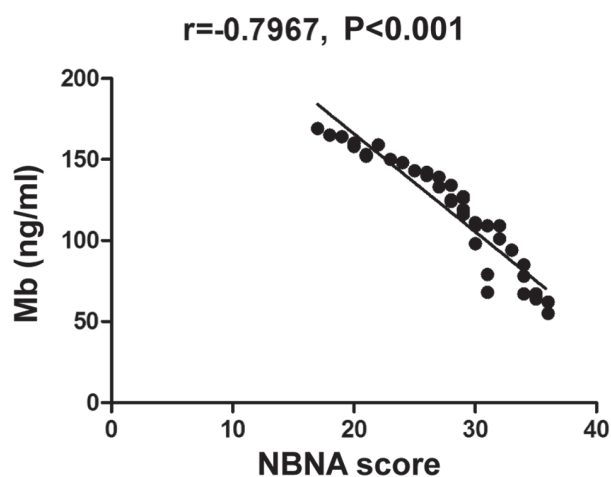


Figure 5. Correlation analysis between NBNA score and umbilical cord blood Mb level. NBNA, neonatal behavioral neurological assessment; Mb, myoglobin.

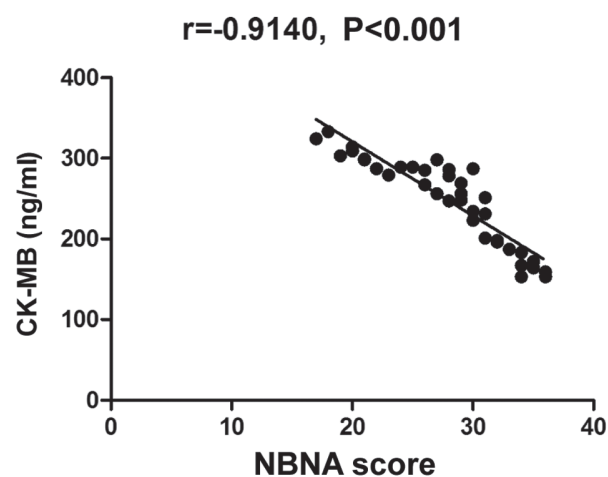


Figure 6. Correlation analysis between NBNA score and umbilical cord blood CK-MB level. NBNA, neonatal behavioral neurological assessment; CK-MB, creatine kinase-MB.

be different degrees of neurological sequelae even if they survive, seriously affecting the prognosis of patients (9). NHIE is considered as a disease seriously threatening the quality of life and even life health of neonates, as well as one of the most important complications of perinatal asphyxia (10). Studies have proved that the main causes of NHIE are maternal factors (11), such as pregnancy-induced hypertension, anemia during pregnancy, postpartum hemorrhage and placental abnormalities (12) (placenta previa, placental abruption and placental dysfunction), as well as fetal factors (13), such as intrauterine hypoxia and developmental malformation. The

above high-risk factors have been clinically recognized, but not only central nervous system injury but also multiple organ dysfunctions occur in NHIE, especially common in the kidneys, cardiovascular system and respiratory system, which then lead to changes in various inflammatory factors, nerve injury-related factors and myocardial enzymes *in vivo*. Therefore, the early effective diagnosis and treatment of NHIE is of important significance for reducing the mortality rate and improving the prognosis of child patients (14).

In the present study, the levels of serum S-100 β , CRP and Cys-C, umbilical cord blood TnI, Mb and CK-MB in NHIE

Table VI. Multivariate logistic regression analysis for risk factors for NHIE.

	β	SE	W	OR	P-value	95% CI
Increased level of S-100 β	0.89	0.53	9.47	2.44	<0.001	1.38-4.31
Increased level of CRP	1.83	0.57	4.82	1.96	0.04	1.03-7.45
Increased level of Cys-C	1.71	33.60	7.11	5.52	<0.001	3.10-9.83
Increased level of TnI	2.92	0.54	4.46	6.86	0.04	1.59-9.24
Increased level of Mb	1.12	5.50	6.38	3.07	<0.001	1.76-5.34
Increased level of CK-MB	2.34	0.46	9.92	2.81	0.01	1.21-4.40

NHIE, neonatal hypoxic ischemic encephalopathy; CRP, C-reactive protein; Cys-C, cystatin C; TnI, troponin I; Mb, myoglobin; CK-MB, creatine kinase-MB.

patients were analyzed. It was found in the comparison of levels of serum S-100 β protein, CRP and Cys-C between the two groups that their levels in observation group were significantly higher than those in control group, indicating that the levels of serum S-100 β protein, CRP and Cys-C in NHIE patients are significantly higher than those in normal children. The comparison of the levels of umbilical cord blood TnI, Mb and CK-MB between the two groups showed that their levels in observation group were also significantly higher than those in control group, suggesting that the levels of umbilical cord blood TnI, Mb and CK-MB obviously rise in NHIE patients. At the same time, the correlations between NBNA score and changes in serum S-100 β protein, CRP and Cys-C, and umbilical cord blood TnI, Mb and CK-MB were analyzed, and the results revealed that the NBNA score was negatively correlated with the changes in serum S-100 β protein, CRP and Cys-C as well as the umbilical cord blood TnI, Mb and CK-MB, further suggesting that with the increasing severity of nervous system dysfunction and disease, the levels of nerve injury-related factors and inflammation are higher and the myocardial damage is more severe in NHIE patients. Finally, the related and independent risk factors for NHIE were analyzed. The results manifested that the levels of serum S-100 β protein, CRP and Cys-C, and umbilical cord blood TnI, Mb and CK-MB were related risk factors for NHIE. The increased levels of serum S-100 β protein, CRP and Cys-C, and umbilical cord blood TnI, Mb and CK-MB were independent risk factors for NHIE.

In neonatal hypoxia-ischemia, S-100 β protein enters the blood through the blood-brain barrier, increasing the level of serum S-100 β protein. In particular, after central nervous system injury definitely occurs in NHIE, the blood-brain barrier is further destroyed, so the level of serum S-100 β protein significantly increases (15). At the same time, the level of Cys-C is also increased, and it has a certain correlation with the local cerebrovascular stimuli in the central nervous system (16), which will aggravate vasospasm (17) and metabolic disorders in the body, induce microcirculation disturbance and platelet aggregation, worsen ischemia and hypoxia in brain tissues, and lead to the increase of CRP level (18). In addition, when asphyxia occurs, there will be obvious hypoxic-ischemic changes in the central nervous system, and the aerobic metabolism is replaced with anaerobic glycolysis to cause massive accumulation of lactic acid, thereby

resulting in myocardial injury, competitive inhibition of intracellular calcium iononproteases and excitation-contraction coupling (19), and aggravating the degradation of binding troponin in the cytoplasm. In addition, myocardial injury is further aggravated due to the effect of lipid peroxides, so the levels of umbilical cord blood TnI, Mb and CK-MB all obviously rise (20).

In conclusion, in NHIE patients, the levels of serum S-100 β protein, CRP and Cys-C, and umbilical cord blood TnI, Mb and CK-MB all significantly rise, and they have negative correlations with the nervous system function after onset.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

BW and GZ designed the study and performed the experiments, BW and XP established the animal models, GZ and JM collected the data, YL and JL analyzed the data, BW and GZ prepared the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Hospital Attached to the Binzhou Medical University (Binzhou, China). Signed informed consents were obtained from the parents and/or guardians of the child participants before the study.

Patient consent for publication

Not available.

Competing interests

The authors declare no competing interests.

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