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Original Article

The Application Value of Combined Detection of Serum IL-6, LDH, S100, NSE, and GFAP in the Early Diagnosis of Brain Damage Caused by Neonatal Asphyxia

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Abstract

Background: We aimed to investigate the correlation and clinical significance between a group of serum biomarkers and brain damage caused by neonatal asphyxia, and to provide sensitive and effective detection methods for early diagnosis and prognosis improvement.

Methods: We enrolled neonates hospitalized in the neonatal department of The Affiliated Hospital of Inner Mongolia Medical University of China from June 2020 to June 2021 as the study subjects. The levels of interleukin-6 (IL-6), lactate dehydrogenase (LDH), S100 protein, neuron-specific enolase (NSE), and glial fibrillary acidic protein (GFAP) in serum samples were measured using electrochemiluminescence (ECL), enzyme-linked immunosorbent assay (ELISA) or rate method and the correlations between these serum biomarkers and the degree of neonatal asphyxia and brain damage were statistically analyzed using Spearman test.

Results: The levels of serum IL-6, LDH, S100, NSE, and GFAP in the neonatal asphyxia with brain damage group within 12 hours after birth were significantly higher than those in the neonatal asphyxia without brain damage group (all $P<0.05$). Additionally, these levels were positively correlated with the degree of asphyxia. The Area Under the Curve (AUC) of receiver operating characteristic (ROC) curves of IL-6 (0.8819), LDH (0.8108), S100 (0.8719), NSE (0.8719), and GFAP (0.8073) were revealed.

Conclusion: The combined detection of serum marker levels can simultaneously reflect neuronal injury, glial cell injury, and inflammatory injury, improve the accuracy of diagnosis of neonatal asphyxia with brain damage, and enable the formulation of treatment strategies as early as possible to reduce the incidence of complications of brain damage.

Keywords: Neonate asphyxia; Brain damage; Early diagnosis; Serum marker

Introduction

Neonatal asphyxia is a severe pathological condition that occurs when various factors disrupt the exchange of gases between the mother and fetus, resulting in oxygen deprivation and the inability

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to establish and maintain normal breathing after birth, which can lead to cerebral hypoxia (1). The neonatal brain has a high metabolic rate and requires significant oxygen for proper functioning. As a result, brain cells become more sensitive without oxygen, resulting in cell swelling, apoptosis, and, ultimately, brain damage (2). Brain damage caused by neonatal asphyxia frequently results in cerebral palsy and intellectual disability and is a leading cause of neonatal mortality worldwide (3). Therefore, early detection of brain damage caused by neonatal asphyxia holds significant clinical and social importance.

In addition to the clinical history, physical and neurological examination, imaging is important in diagnosing brain damage caused by neonatal asphyxia. Compared to cranial ultrasound and computed tomography, magnetic resonance imaging (MRI) is commonly used in diagnosing neonatal brain damage (4). Yet, using MRI for bedside quantitative analysis, predicting complications, and determining the type of damaged cells and brain function status, is still changeling (5). Since it can be very challenging to early assessment of the risk of brain damage caused by neonatal asphyxia, it is crucial to identify facile and accurate biomarkers for early detection of brain damage caused by neonatal asphyxia that would enable prompt imaging assessment and therapy, thereby preventing the progression of brain damage (6).

Over the years, several serum biochemical markers have been proposed for diagnosing neonatal brain damage (7). Compared to other diagnostic methods, they can be assessed immediately after birth, which can compensate for the limitations of imaging examination methods. However, there are still several shortcomings in the current clinical application of single serum markers to predict brain damage caused by neonatal asphyxia

We aimed to assess the correlation and clinical significance between a group of serum biomarkers (i.e., NSE, IL-6, S100 protein, LDH, and GFAP) and brain damage caused by neonatal asphyxia and to provide sensitive and effective detection methods for early diagnosis and prognosis improvement of brain damage caused by neonatal asphyxia.

Materials and Methods

Study subjects

The study focused on consecutively hospitalized neonates in the Neonatal Department of The Affiliated Hospital of Inner Mongolia Medical University of China from June 2020 to June 2021 (subjects admitted 12 hours after birth). The neonates were divided into two groups, the non-asphyxia group and the asphyxia group, based on their 1-minute Apgar score and umbilical cord blood pH value. The asphyxia group was further divided into two subgroups, the mild asphyxia group and the severe asphyxia group, based on their 1-minute Apgar score and umbilical cord blood pH value. The asphyxia group was then divided into two subgroups, the asphyxia with brain damage and the asphyxia without brain damage, based on clinical manifestations and imaging changes.

Grouping of study subjects and inclusion criteria

The inclusion criteria for the neonatal asphyxia group were: (1 neonates who had high-risk factors during the prenatal, perinatal, or fetal period that could lead to asphyxia; (2 neonates who failed to establish effective spontaneous breathing at 1 minute after standard resuscitation measures and had an Apgar score ≤ 7, with umbilical artery blood pH <7.2; other possible causes of low Apgar score had been ruled out; inclusion criteria for the neonatal asphyxia with brain damage group were: (a) neonates with clinical manifestations of brain damage, such as central respiratory arrest, blood pressure fluctuations, altered consciousness, seizures, abnormal muscle tone, etc.; (b) neonates with imaging or neurophysiological changes indicative of brain damage, such as cerebral white matter softening, cerebral edema, intracranial hemorrhage, severe ventricular dilation, abnormal discharge on electroencephalogram, etc.; inclusion criteria for neonatal asphyxia without brain damage group were: (a)

neonates without clinical manifestations, imaging changes, or neurophysiological changes indicative of brain damage. The exclusion criteria for the neonatal asphyxia group were: congenital malformations, chromosomal abnormalities, and inherited metabolic diseases; neonates born to mothers with myocardial disease, endocrine disorders, chorioamnionitis, or hypertension. Classification of neonatal asphyxia: (1 neonates with 1-minute Apgar scores of 1-3 and umbilical artery blood pH < 7.0 were classified as severe asphyxia; (2 neonates with 1-minute Apgar scores of 4-7 and umbilical artery blood pH < 7.2 were classified as mild asphyxia.

Inclusion criteria for the non-asphyxia group were: (1 neonates diagnosed with conditions such as meconium aspiration, wet lung, or high-risk neonates and admitted within 12 hours after birth; (2 non-asphyxia newborns with 1-minute and 5-minute Apgar scores of 8-10 were also included in the study. The exclusion criteria for the non-asphyxia group were: newborns with congenital malformations, chromosomal abnormalities, inherited metabolic diseases, and other diseases.

This study complies with the Helsinki Declaration and has been approved by the Ethics Committee of The Affiliated Hospital of Inner Mongolia Medical University (NO.YKD202001146).

Sample Collection and Testing

Blood samples were collected within 12 hours after birth and during the first hospitalization by the laboratory department. The samples were centrifuged at 3000g/minute for 10 minutes, and the levels of serum NSE, IL-6, S100 protein, and LDH were measured using Roche Cobas8000 fully automated biochemical analyzer. The NSE, IL-6, and S100 levels were measured using electrochemiluminescence immunoassay, and the LDH level was measured using the rate method. The remaining serums were stored at -80℃, and GFAP levels were measured using ELISA.

Statistical analyses

Data statistical analysis was performed using SPSS-21.0 (IBM Corp., Armonk, NY, USA). The mean ± standard error (x±SE) was used to represent quantitative data, and $p < 0.05$ indicated statistical significance. The chi-square test was used to compare the gender distribution between the patients of the two groups. The Mann-Whitney U test was used to compare the levels of gestational age, birth weight, S100, NSE, LDH, IL-6, and GFAP between the patients of the two groups. The Spearman correlation analysis was used to investigate the correlation between the pH value of umbilical artery blood, the 1-minute Apgar score, and the serum biomarkers of brain damage, including S100, NSE, LDH, IL-6, and GFAP. ROC curve analysis was conducted to assess the diagnostic accuracy of GFAP, LDH, IL-6, NSE, and S100 levels in neonates' serum to detect brain damage caused by neonatal asphyxia.

Results

General information

We included 129 neonates. Based on the inclusion criteria, the neonates were categorized into two groups: the non-asphyxia group (n=61) and the asphyxia group (n=68). As indicated in Table 1, there were no significant differences in terms of gender, gestational age, and birth weight between the two groups.

Impact of serum GFAP, LDH, IL-6, NSE, and S100 levels on neonatal asphyxia

Table 2 shows that the serum levels of S100, NSE, LDH, IL-6, and GFAP were significantly higher in the asphyxia group compared to the non-asphyxia group (all $P < 0.05$). These findings suggest that the level of these brain damage-associated serum biomarkers can be increased by asphyxia, and asphyxia may lead to brain damage in neonates.

Table 1: Comparison of general clinical data between neonates in the non-asphyxia group and asphyxia group

Characteristic	*Non-asphyxia (n=61)*	*Asphyxia (n=68)*	*P-value*
male (number)	31	42	0.2105
female (number)	30	26	
gestational age (weeks)	35.12 ± 0.39	34.05 ± 0.49	0.0529
weight at birth (g)	2260.98 ± 83.20	2175.22 ± 114.36	0.0540

Table 2: Comparison of serum levels of GFAP, LDH, IL-6, NSE, and S100 between neonates in the non-asphyxia group and asphyxia group

Item	*Non-asphyxia*	*Asphyxia*	*P-value*
GFAP (ng/L)	0.82 ± 0.04	1.36 ± 0.06*	0.0001
LDH (U/L)	466.60 ± 13.9	799.63 ± 62.01*	0.0001
IL-6 (pg/ml)	78.04 ± 11.5	458.80 ± 91.08*	0.0001
NSE (μg/L)	34.73 ± 1.41	70.79 ± 4.00*	0.0001
S100 (μg/L)	0.83 ± 0.04	2.61 ± 0.46*	0.0001

* Statistically significant difference ($P < 0.05$) when compared to non-asphyxia group.

Impact of serum GFAP, LDH, IL-6, NSE, and S100 levels on neonatal asphyxia severity

The 68 neonates in the asphyxia group were further divided into the mild asphyxia group (n=53) and severe asphyxia group (n=15). Compared with the mild asphyxia group, the serum levels of S100, NSE, LDH, IL-6, and GFAP in the severe asphyxia group were significantly increased, with statistically significant differences (all $P < 0.05$), as shown in Table 3, which suggests that the severity of asphyxia may worsen the degree of brain damage in neonates.

Table 3: Comparison of serum levels of GFAP, LDH, IL-6, NSE, and S100 between neonates in the mild asphyxia group and severe asphyxia groups

Item	*Mild asphyxia (n=53)*	*Severe asphyxia (n=15)*	*P-value*
GFAP (ng/L)	1.23 ± 0.05	1.79 ± 0.17*	0.0026
LDH (U/L)	679.71 ± 27.35	1223.33 ± 239.09*	0.0012
IL-6 (pg/ml)	122.11 ± 41.77	1212.46 ± 321.51*	0.0001
NSE (μg/L)	65.54 ± 3.74	88.28 ± 11.12*	0.0323
S100 (μg/L)	1.72 ± 0.13	3.73 ± 0.61*	0.0001

* Statistically significant difference ($P < 0.05$) when compared to mild asphyxia group

The 1-minute Apgar score and umbilical artery blood pH value are the standard measures to assess the severity of neonatal asphyxia. *Spearman* correlation analysis showed that both the 1-minute Apgar score and umbilical artery blood pH value were negatively correlated with the serum levels of S100, NSE, LDH, IL-6, and GFAP ($P<0.05$; Tables 4 and 5). These findings further

support the notion that the serum levels of S100, NSE, LDH, IL-6, and GFAP are associated with the severity of asphyxia and that the severity of asphyxia may exacerbate the degree of brain damage in neonates.

Table 4: Correlation analysis between 1-minute Apgar score and serum levels of GFAP, LDH, IL-6, NSE, and S100 in neonates.

Item	*r Value*	*95%CI*	*P-value*
GFAP	-0.4094	-0.6104--0.1588	0.0016
LDH	-0.3863	-0.5809--0.1500	0.0015
IL-6	-0.3819	-0.5735--0.1508	0.0013
NSE	-0.4915	-0.6575--0.2800	0.0001
S100	-0.5606	-0.7146--0.3548	0.0001

Table 5: Correlation analysis between umbilical artery blood pH value and serum levels of GFAP, LDH, IL-6, NSE, and S100 in neonates.

Item	*r Value*	*95%CI*	*P-value*
GFAP	-0.4992	-0.6766--0.2669	0.0001
LDH	-0.3589	-0.5553--0.1126	0.0027
IL-6	-0.3978	-0.5859--0.1691	0.0008
NSE	-0.4258	-0.6113--0.1958	0.0004
S100	-0.6769	-0.7955--0.5085	0.0001

Impact of serum GFAP, LDH, IL-6, NSE, and S100 levels on brain damage induced by asphyxia

The study divided the asphyxia group into two subgroups based on clinical manifestations and imaging changes: the asphyxia with brain damage group (n=36) and the asphyxia without brain damage group (n=32). Table 6 shows that the serum levels of S100, NSE, LDH, IL-6, and GFAP were significantly higher in the asphyxia with brain damage group compared to the asphyxia without brain damage group (all $P < 0.05$). These findings provide further evidence that brain damage caused by neonatal asphyxia can significantly increase the serum levels of S100, NSE, LDH, IL-6, and GFAP in neonates.

Table 6: Comparison of serum levels of GFAP, LDH, IL-6, NSE, and S100 between asphyxia without brain damage group and asphyxia with brain damage group.

Item	*asphyxia without brain damage (n=36)*	*asphyxia with brain damage (n=32)*	*P-value*
GFAP (ng/L)	1.11 ± 0.06	1.62 ± 0.08*	0.0001
LDH (U/L)	617.02 ± 29.32	1005.06 ± 118.31*	0.0001
IL-6 (pg/ml)	127.55 ± 23.38	831.47 ± 170.14*	0.0001
NSE (μg/L)	52.86 ± 2.76	90.46 ± 6.16*	0.0001
S100 (μg/L)	1.37 ± 0.09	3.77 ± 0.85*	0.0001

* Statistically significant difference ($P< 0.05$) when compared to asphyxia without brain damage group.

ROC curve analysis was performed to assess the diagnostic value of serum GFAP, LDH, IL-6, NSE, and S100 levels in diagnosing brain damage caused by neonatal asphyxia. The results indicated that all five biomarkers had diagnostic values for asphyxia-induced brain damage, and the cut-off values for diagnosing brain damage caused by neonatal asphyxia were calculated and presented in Table 7.

Table 7: Diagnostic value and efficacy of serum GFAP, LDH, IL-6, NSE, and S100 levels in diagnosing brain damage caused by neonatal asphyxia

Item	*Cutoff*	*Sensitivity (%)*	*Specificity (%)*	*AUC*	*Standard error*	*95%CI*	*P-value*
GFAP	1.167 ng/L	96.43	58.62	0.8073	0.05755	0.6944-0.9201	0.0001
LDH	687.5 U/L	75	77.78	0.8108	0.05088	0.7110-0.9105	0.0001
Il-6	154.4 pg/ml	90.63	77.78	0.8819	0.04145	0.8007 - 0.9632	0.0001
NSE	62.47μg/L	87.1	79.41	0.8719	0.04505	0.7836-0.9602	0.0001
S100	1.825μg/L	75	86.67	0.8719	0.04359	0.7864-0.9573	0.0001

Discussion

Brain damage caused by neonatal asphyxia is a common cause of disability and mortality among newborns. While imaging is a very useful tool for detecting brain damage in adults, this diagnostic approach is more challenging to perform in neonates. Serum biochemical marker detection can compensate for the imaging, but its diagnostic efficacy is still controversial. Therefore, further studies are necessary to understand fully its potential value in diagnosing brain damage caused by neonatal asphyxia. This study investigated the diagnostic value of detecting serum biomarkers GFAP, LDH, IL-6, NSE, and S100 protein expression levels, both individually and in combination, for brain damage caused by neonatal asphyxia. The study yielded several key findings. Firstly, it was observed that asphyxia could significantly increase the serum levels of brain damage biomarkers within 12 hours of birth. Secondly, the severity of asphyxia was positively correlated with the serum levels of these biomarkers. Thirdly, the study found that the serum levels of brain damage biomarkers in the asphyxia with brain damage group were significantly increased when compared to the asphyxia without brain damage group. This further confirms that brain damage caused by neonatal asphyxia can significantly increase the serum levels of biomarkers, which has clinical significance. Fourthly, the study conducted ROC curve analysis to assess the diagnostic value of serum biomarker levels in detecting brain damage caused by neonatal asphyxia. The results demonstrated that all five biomarkers had significant diagnostic value for asphyxia-induced brain damage.

IL-6 could also serve as a brain damage biomarker in animal experiments and clinical tests (8, 9). The findings of this study are consistent with previous research, demonstrating a significant increase in serum IL-6 levels in newborns with asphyxia and asphyxia-induced brain injury. This study provides evidence that serum IL-6 levels have strong diagnostic value for brain damage caused by neonatal asphyxia.

LDH, a biomarker commonly used for myocardial assessment in clinical practice, has been found to have the potential as a biomarker for brain

injury (10-12). However, further clinical studies are needed to evaluate the diagnostic value of serum LDH in cases of brain damage caused by neonatal asphyxia. In this study, serum LDH levels increase significantly in cases of brain damage caused by neonatal asphyxia, which is consistent with the results of the studies above. The study also suggests that LDH has diagnostic value for brain damage caused by neonatal asphyxia, with an area under the ROC curve of 0.8108. In addition, the LDH rate method detection is relatively inexpensive and can be performed using a standard biochemical analyzer or even a spectrophotometer. This makes it a more practical option than IL-6, S100, and NSE detection in economically underdeveloped areas, and it has the potential to be promoted in primary hospitals.

S100 protein is a dimeric acidic calcium-binding protein primarily found in the central nervous system astrocytes (13). S100 was associated with neonatal intellectual development and has diagnostic and prognostic value in cases of neonatal hypoxic-ischemic encephalopathy (14). Irmak and colleagues (15) discovered a negative correlation between S100 umbilical cord blood levels and 1-minute and 5-minute Apgar scores. However, there are issues with the quality control of umbilical cord blood prior to testing, which makes it difficult to conduct prognostic monitoring (16, 17). As a result, most laboratories only perform venous blood testing and other related procedures. Hence, in our study, venous blood from newborns was utilized to detect S100, which ensured superior quality control and facilitated prompt sampling and testing.

GFAP is predominantly found in astrocytes within the central nervous system, and its levels can significantly elevate in the blood and cerebrospinal fluid of patients with various subtle injuries of the central nervous system (18-20). Nonetheless, researcher have reported (21) that when CT results are used as the inclusion criteria for traumatic brain injury patients, the sensitivity and specificity of serum GFAP levels in diagnosing traumatic brain injury are low. Due to the conflicting results regarding the diagnostic efficacy of GFAP for brain damage, this study aimed to verify its diagnostic potential for brain damage caused by neonatal asphyxia. We found that serum GFAP levels were significantly elevated in brain damage caused by neonatal asphyxia, indicating that GFAP levels could serve as a potential diagnostic biomarker for this condition. However, the diagnostic value of GFAP levels was found to be relatively moderate in terms of specificity.

NSE has been a traditional biomarker for brain damage widely used in clinical practice (22). However, NSE is susceptible to interference from factors such as hemolysis and jaundice (23). Newborns, especially premature and low birth weight neonates, are a special population, and blood collection can be extremely challenging, often leading to specimen hemolysis. Therefore, it is essential to test other biomarkers for brain damage in combination to avoid interference. Hemolysis and jaundice have little effect on IL-6 and S100, which can effectively solve the problem of interference caused by hemolysis and jaundice. However, detecting brain damage in neonates at an early stage continues to be a persistent challenge. Despite advancements in perinatal monitoring techniques, the occurrence of brain injury in neonates has not shown a substantial decline (24). In this context, identifying serum biomarkers specific to brain damage holds significant importance in facilitating early diagnosis and intervention.

Conclusion

IL-6, LDH, S100, NSE, and GFAP within 12 hours of birth serve as reliable biomarkers for brain damage in cases of neonatal asphyxia and asphyxia-induced brain damage. These biomarkers are significantly elevated and positively correlated with the severity of asphyxia and possess diagnostic value for identifying neonatal asphyxia-induced brain damage. The combination of these five biomarkers reinforces their diagnostic accuracy and provides a comprehensive assessment of neuronal injury, glial cell injury, and inflammatory injury simultaneously. This not only enhances diagnostic efficiency and prevents

misdiagnosis but also offers a more complete understanding of the extent and nature of the brain damage. It effectively solves the problem of interference caused by hemolysis and jaundice and provides new ideas for balancing diagnostic efficiency and economic cost control. In conclusion, the study suggests that serum levels of IL-6, LDH, S100, NSE, and GFAP have good diagnostic value for brain damage caused by neonatal asphyxia. Moreover, combining these five biomarkers can enhance diagnostic efficiency and specificity. These findings provide valuable scientific evidence for clinicians to reflect on the degree of brain damage caused by neonatal asphyxia and the occurrence of warning complications.

Journalism Ethics considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflict of interest

All authors declare no conflict of interest.

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