Journal of Clinical and Nursing Research, 2025, Volume 9, Issue 7
http://ojs.bbwpublisher.com/index.php/JCNR
Online ISSN: 2208-3693
Print ISSN: 2208-3685

Research Progress on Exploring Biomarkers for Early Diagnosis of Neonatal Hypoxic-Ischemic Encephalopathy Based on Proteomics Technology

Zhaojun Geng, Yumei Liang*

Youjiang Medical University for Nationalities, Baise 533000, Guangxi, China

**Corresponding author: Yumei Liang, LYM8591@163.com*

Abstract: Neonatal hypoxic-ischemic encephalopathy (HIE) refers to neonatal brain damage caused by various factors during the perinatal period that lead to hypoxia and reduced cerebral blood flow [1]. Globally, 0.2% to 2.26% of newborns develop HIE, with approximately 20% resulting in neonatal death and about 25% of survivors suffering from neurological impairment [2]. Currently, there is a lack of highly sensitive and specific diagnostic tools for HIE, posing significant challenges to reducing HIE mortality and neurological abnormalities [3]. The development of high-throughput proteomics technology based on mass spectrometry (MS) has significantly enhanced the potential to discover biomarkers in biological fluids such as plasma, cerebrospinal fluid, saliva, and urine [4]. Proteomics technology has become an engine for exploring novel markers of HIE [5]. This article systematically reviews the progress of proteomics technology in the study of biomarkers for the early diagnosis of HIE, elucidating its potential application value.

Keywords: Neonatal hypoxic-ischemic encephalopathy; Proteomics; Biomarkers

Online publication: August 8, 2025

1. IntroductionNeonatal hypoxic-ischemic encephalopathy (HIE) is a severe brain injury caused by perinatal hypoxia and reduced cerebral blood flow, affecting 0.2%–2.26% of newborns globally. Despite its high mortality (~20%) and risk of neurological sequelae (25% of survivors), current diagnostic tools lack sufficient sensitivity and specificity. Advances in mass spectrometry (MS)-based proteomics offer promising opportunities to discover HIE biomarkers in biofluids (e.g., plasma, cerebrospinal fluid), potentially transforming early diagnosis and outcomes. This review highlights the progress and clinical potential of proteomics in HIE biomarker research.

2. Development of proteomics technology

The human proteome, as the final product of gene expression, plays a central role in the physiological and pathological processes of organisms. It covers all proteins expressed in every cell, tissue, and organ of the human body, serving as a bridge between genotype and phenotype [6]. Proteomics involves the process of identifying and quantifying proteins, as well as determining their localization, composition, structure, function, interactions, expression profiles, and modifications. It holds significant importance in biomedical research, such as deciphering disease pathogenesis, prognosis, and diagnosis [4].

In recent years, many studies have investigated and validated disease pathogenesis and markers using proteomic methods. Mass spectrometry (MS)-based proteomics technology, with its high-precision and high-sensitivity protein quantification capabilities, has become the core of protein identification. It mainly consists of three key steps: protein separation, identification, and validation [7]. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) technology, with its advantages of high throughput and sensitivity, is the mainstream method for protein separation [8].

Quantitative proteomics techniques are mainly divided into non-targeted relative quantification and targeted absolute quantification. The former is further categorized into label-free quantification and labeling quantification techniques. Commonly used label-free quantification techniques include Label-free quantification and Data-Independent Acquisition (DIA), while labeling quantification techniques cover chemical labeling, Stable Isotope Labeling by Amino acids in Cell culture (SILAC), Isobaric Tags for Relative and Absolute Quantitation (iTRAQ), and Tandem Mass Tags (TMT). The latter mainly includes Isotope Dilution Mass Spectrometry (IDMS), Multiple Reaction Monitoring (MRM), and Parallel Reaction Monitoring (PRM) [9]. Protein validation methods include Enzyme-Linked Immunosorbent Assay (ELISA), immunoblotting, and immunohistochemistry [7].

Previously, high-abundance proteins obscured low-abundance potential disease markers. However, with the rapid development of high-throughput proteomics technology, the depth of coverage, accuracy, and efficiency of proteome analysis have significantly improved. Potential diagnostic markers efficiently screened based on proteomics technology will have higher clinical diagnostic and predictive value [6].

3. Current research status of HIE protein markers

Currently, there are six main categories of biomarkers for brain injury: neuronal specific injury markers, axonal integrity markers, astrocyte injury markers, demyelination markers, blood-brain barrier function indicators, and oxidative stress products [10]. Among them, the following protein markers are worthy of attention:

3.1. Neuron-specific injury markers

3.1.1. Neuron-specific Enolase (NSE)

NSE is a cell-specific subtype of the glycolytic enzyme enolase, which mainly exists in mature neurons and neuroendocrine cells and is commonly used as a clinical marker for neurons and neuroendocrine cells [10]. Studies have shown that there is no significant difference in NSE levels among different groups of HIE patients on the first day after birth; however, NSE levels on the third day have high sensitivity and specificity for predicting neurodevelopmental outcomes [11]. Traditional detection techniques can be affected by high-abundance proteins, which can interfere with the detection of low-abundance proteins and reduce detection throughput, thus obscuring important protein information [6]. With the development of proteomics technology, it may be possible to more

accurately detect changes in NSE concentration in the blood and more sensitively identify early disease-related subtle changes, thereby improving the sensitivity of NSE as an early diagnostic biomarker [12].

3.1.2. Ubiquitin carboxy-terminal hydrolase L1 (UCH-L1)

UCH-L1 is a deubiquitinating enzyme that regulates target protein stability by modulating target protein degradation and cleaving attached polyubiquitin chains, primarily in neurons and neuroendocrine cells in the brain [10]. Researchers such as Mahmut Ok have demonstrated for the first time in a perinatal asphyxia calf model that the synergistic increase of UCHL1 and S100B can specifically reflect hypoxic-ischemic brain damage [13]. Clinical evidence shows that the level of UCH-L1 in the serum of children with HIE increases significantly 6 hours after birth. It can be used for the diagnosis of acute brain injury caused by HIE and reflects the severity of HIE [14].

3.1.3. Brain-derived neurotrophic factor (BDNF)

BDNF is a secreted protein molecule widely distributed in the nervous system. This protein is mainly involved in regulating key physiological processes such as neuronal development, survival, differentiation, and synaptic plasticity through the signal pathway mediated by the TrkB receptor [15]. Through high-throughput proteomics analysis, researchers such as Sumrati Gurtoo compared 51 differentially expressed proteins found in the serum of children with HIE and discovered that BDNF increased the most significantly. It is speculated that blood BDNF can be used as a biochemical indicator for early diagnosis of the severity of HIE [16].

3.2. Markers of axonal integrity

3.2.1. Neurofilament Light Chain Protein (NFL)

Neurofilaments, as a core component of the neuronal cytoskeleton, play a key role in maintaining the structural stability of neurons. NFL is the most abundant component of neurofilament proteins and is a biomarker of axonal damage in various neurodegenerative diseases [17]. Limited by the sensitivity bottleneck of traditional ELISA technology, blood NFL detection has not been achieved for a long time. With the development of proteomics technology, Single Molecule Array (SiMoA) technology has a sensitivity more than 1000 times that of ELISA technology. Currently, several studies have confirmed that SiMoA technology can reliably detect NFL in blood samples [18]. Research shows that the serum concentration of NFL increases significantly within 0–6 hours after birth in neonates with HIE, and the increase is more significant in the moderate to severe HIE group, indicating its potential application value in early identification of HIE [19].

3.2.2. Tau protein

Tau is an important structural element of the axonal cytoskeleton. Its primary function is to maintain the stability of microtubules and coordinate the movement of molecules along them. Its physiological function is strictly regulated by phosphorylation. Many factors can alter the activity of kinases or phosphatases through multiple signal transduction pathways, triggering excessive phosphorylation of Tau, which leads to microtubule dysfunction and neuronal cell death [10]. Due to the heterogeneity and dynamic modification characteristics of tau protein subtypes, previous detection techniques have struggled to improve the sensitivity of early diagnosis. However, breakthroughs in ELISA detection technology have enabled the detection sensitivity of Tau protein to reach the fg/μL level [20]. Studies have shown that the sensitivity of serum Tau protein levels within 24 hours of birth to predict neurodevelopmental delay reaches 100%, suggesting that it can serve as a key indicator for early diagnosis and

prognosis evaluation of HIE [21].

3.3. Markers of astrocyte injury

3.3.1. Glial Fibrillary Acidic Protein (GFAP)

GFAP is an acidic cytoskeletal protein primarily expressed in astrocytes. Its main physiological functions are to maintain the morphology and stability of astrocytes while promoting material transfer and exchange between glial cells in the CNS. Brain injury and related inflammatory responses activate astrocytes and stimulate their proliferation in damaged areas. The high expression of GFAP makes it a promising biomarker for the diagnosis, prognosis, and treatment of nerve injury and other neurological diseases [10]. Traditional ELISA methods can only detect GFAP at the ng/mL level. However, with breakthroughs in detection technology, SiMoA technology has improved detection sensitivity to 16.6 pg/mL through a single-molecule counting strategy [22]. The FDA has approved the combined use of UCH-L1 and GFAP biomarkers in blood to aid in the diagnosis of patients with mild brain injury [17].

3.4. Demyelination markers

3.4.1. Myelin Basic Protein (MBP)

MBP is a structural myelin protein located on the surface of the myelin sheath. It helps maintain myelin stability and plays a crucial role in initiating myelin formation in nerve cells. Studies have confirmed that the more severe the brain injury, the greater the myelin breakdown, and the higher the degree of blood-brain barrier (BBB) damage, leading to increased long-term release of MBP into the bloodstream from neurons. Detecting MBP concentration in serum can help assess the extent of brain injury [10]. Research has found that MBP expression in rat brain tissue decreases significantly 1–3 days after hypoxia-ischemia, suggesting that changes in its expression can early reflect myelin damage. Serum MBP levels may be an indicator for assessing brain injury in newborns with HIE [23].

3.5. Blood-brain barrier function indicators

3.5.1. Matrix Metalloproteinase-9 (MMP-9)

MMP-9 is a member of the matrix metalloproteinase family, MMPs. By degrading tight junction proteins (TJPs) between endothelial cells of the blood-brain barrier (BBB) and disrupting the extracellular matrix, it increases BBB permeability and participates in the pathological processes of various neurological diseases [24]. Studies have confirmed that MMP-9 is a key factor in disrupting the blood-brain barrier during hypoxic-ischemic brain injury in newborns, reaching peak activity within 24 hours after brain injury. It can serve as a biomarker for early diagnosis of HIE [25].

3.6. Products of oxidative stress

3.6.1. Heat Shock Protein 70 (HSP70)

HSP70 is a crucial member of the heat shock protein (HSP) family, which under normal conditions participates in the pathogenesis of various diseases through anti-apoptotic activity, protecting cells and maintaining homeostasis [26]. As a sensitive biomarker for asphyxia, it is a major stress protein induced by cerebral ischemia. Studies have shown that HSP70 is significantly elevated in serum samples taken within 6 hours after birth from neonates with severe HIE [27].

4. Conclusion

Although proteomics research has provided a new direction for the early diagnosis of HIE, this field is still in its developmental stage. The advancement of proteomics technology has enabled the identification of a large number of potential markers, offering possibilities for early HIE diagnosis. However, their clinical translation requires systematic validation. By advancing the combination of proteomics technology development and clinical validation, and screening for highly specific markers related to HIE, it is hoped that scientific evidence can be provided for the early diagnosis of HIE in infants, reducing mortality and morbidity rates, and ultimately improving the long-term prognosis of these patients.

Disclosure statement

The authors declare no conflict of interest.

References

[1] Sun Y, Cai C, 2021, Research Progress on the Treatment of Neonatal Hypoxic-Ischemic Encephalopathy. Chinese Journal of Applied Clinical Pediatrics, 36(8): 631–634.

[2] Li M, Zhang J, Yu S, et al., 2023, Research Progress of Therapeutic Mild Hypothermia in Moderate to Severe Hypoxic-Ischemic Encephalopathy in Neonates. The Journal of Practical Medicine, 39(18): 2411–2415.

[3] Huang J, Xu X, Zeng L, et al., 2024, Evaluation of Hypothermia Treatment for Neonatal Hypoxic-Ischemic Encephalopathy Based on Amplitude-Integrated Electroencephalogram. Chinese Journal of Medical Physics, 41(8): 1036–1040.

[4] Gobena S, Admassu B, Kinde MZ, et al., 2024, Proteomics and Its Current Application in the Biomedical Area: A Concise Review. The Scientific World Journal, 2024: 4454744.

[5] Zhu Y, Yun Y, Jin M, et al., 2020, Identification of Novel Biomarkers for Neonatal Hypoxic-Ischemic Encephalopathy Using iTRAQ. Italian Journal of Pediatrics, 46: 67.

[6] Xie Y, Chen X, Xu M, et al., 2025, Application of the Human Proteome in Disease, Diagnosis, and Translation into Precision Medicine: Current Status and Future Prospects. Biomedicines, 13(3): 681.

[7] Alharbi RA, 2020, Proteomics Approach and Techniques in Identification of Reliable Biomarkers for Diseases. Saudi Journal of Biological Sciences, 27(3): 968–974.

[8] Kanao E, Ishihama Y, 2025, StageTip: A Little Giant Unveiling the Potential of Mass Spectrometry-Based Proteomics. Analytical Sciences: The International Journal of the Japan Society for Analytical Chemistry, 41(5): 667–675.

[9] Ran B, Liang N, Sun H, 2020, Research Progress on the Exploration of Tumor Protein Markers Based on Mass Spectrometry High-Throughput Proteomics Technology. Chinese Journal of Clinical Oncology, 47(8): 411–417.

[10] Zhang H, Wang J, Qu Y, et al., 2025, Brain Injury Biomarkers and Applications in Neurological Diseases. Chinese Medical Journal, 138(1): 5–14.

[11] Huang HZ, Hu XF, Wen XH, et al., 2022, Serum Neuron-Specific Enolase, Magnetic Resonance Imaging, and Electrophysiology for Predicting Neurodevelopmental Outcomes of Neonates with Hypoxic-Ischemic Encephalopathy: A Prospective Study. BMC Pediatrics, 22(1): 290.

[12] Yates JR, 2019, Recent Technical Advances in Proteomics. F1000Research, 8(F1000 Faculty Rev-351): 351.

[13] Ok M, Naseri A, Ates MB, et al., 2022, The Usefulness of Serum Brain Damage Biomarkers in Detection and Evaluation of Hypoxic Ischemic Encephalopathy in Calves with Perinatal Asphyxia. Animals: An Open Access Journal

from MDPI, 12(22): 3223.

[14] Zeng S, Huang Y, Zhong T, et al., 2021, The Expression and Clinical Value of Ubiquitin Carboxyl-Terminal Hydrolase L1 in the Blood of Neonates with Hypoxic Ischemic Encephalopathy. Translational Pediatrics, 10(8): 2063–2068.

[15] Zhao C, Yang Y, Yang H, 2020, Research Progress on the Role of Brain-Derived Neurotrophic Factor in Neonatal Hypoxic-Ischemic Encephalopathy. Chinese Journal of Perinatal Medicine, 23(10): 690–694.

[16] Gurtoo S, Kotimoole CN, Sahana KS, et al., 2024, Identification of Novel Biomarkers Using Serum and Urinary Proteomics for Early Detection of Hypoxic Ischemic Encephalopathy. Indian Journal of Clinical Biochemistry: IJCB, 39(4): 506–518.

[17] Lisi I, Moro F, Mazzone E, et al., 2025, Exploiting Blood-Based Biomarkers to Align Preclinical Models with Human Traumatic Brain Injury. Brain: A Journal of Neurology, 148(4): 1062–1080.

[18] Long X, Chen Z, Jiang H, 2024, Neurofilament Light Chain Protein and Polyglutamine Diseases. Chinese Journal of Medical Genetics, 41(9): 1131–1138.

[19] Gaulee P, Yang Z, Sura L, et al., 2022, Concentration of Serum Biomarkers of Brain Injury in Neonates with a Low Cord pH with or Without Mild Hypoxic-Ischemic Encephalopathy. Frontiers in Neurology, 13: 934755.

[20] Tabeshmehr P, Eftekharpour E, 2023, Tau; One Protein, So Many Diseases. Biology, 12(2): 244.

[21] Li M, Zhang B, 2018, Research Progress on Serological Markers Related to Hypoxic-Ischemic Brain Damage in Neonates. Chinese Journal of Neonatology (Chinese and English), 33(5): 388–392.

[22] Paul JF, Ducroux C, Correia P, et al., 2024, Serum Glial Fibrillary Acidic Protein in Acute Stroke: Feasibility to Determine Stroke-Type, Timeline, and Tissue-Impact. Frontiers in Neurology, 15: 1470718.

[23] Zhang J, Zhang K, Wu Z, 2020, Study on the Relationship Between Serum MBP and GFAP Levels and the Degree of Brain Damage in Neonatal Hypoxic-Ischemic Encephalopathy. Chinese Journal of Practical Nervous Diseases, 23(22): 1986–1991.

[24] Liu X, Lu Y, Lin D, et al., 2024, Research Progress on the Relationship Between Inflammatory Markers and Prognosis of Patients with Ischemic Stroke. Chinese Journal of Geriatric Cardiology and Cerebrovascular Diseases, 26(1): 113–115.

[25] Salah MM, Abdelmawla M, Eid SR, et al., 2019, Role of Matrix Metalloproteinase-9 in Neonatal Hypoxic-Ischemic Encephalopathy. Open Access Macedonian Journal of Medical Sciences, 7(13): 2114–2118.

[26] Cao J, Yang L, Wang L, et al., 2021, Heat Shock Protein 70 Attenuates Hypoxia-Induced Apoptosis of Pulmonary Microvascular Endothelial Cells Isolated from Neonatal Rats. Molecular Medicine Reports, 24(4): 690.

[27] Seo YM, Hwang-Bo S, Im SA, et al., 2022, Predictive Value of Heat-Shock Protein Gene Expression on Severe Neonatal Hypoxic-Ischemic Encephalopathy. Diagnostics (Basel, Switzerland), 12(4): 981.