Biomarkers of Hypoxic-Ischemic Encephalopathy in Newborns

Mahmood Noorishadkam[1], Shekoofeh Savabieh[1*], Mohammad Emad Sharifi[2]

[1] Mother and Newborn Health Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[2] Dentistry Student, School of Dental Medicine, Tehran University of Medical Sciences, Tehran, Iran

ARTICLE INFO

ABSTRACT

Biomarkers are particles that are released from target organs during tissue hypoxia injury. Recognizing biomarkers released from the damaged brain helps physicians determine the extent of tissue damage and the use of protective techniques in clinical treatment. Previous studies revealed that biomarkers such as brain-specific proteins (neuron-specific enolase (NSE), S100B, ubiquitincarboxy-terminal hydrolase-L1, total Tau) and cytokines, including IL-6, IL-1β, IL-10, IL-13, interferon-gamma, TNF alpha and brain-derived neurotrophic factor are useful in diagnosing hypoxic-ischemic encephalopathy (HIE) and predicting nerve growth outcomes. However, optimal sensitivity and specificity of these biomarkers have not been achieved, which has limited their clinical application. This review focuses on biomarkers such as lactate, LDH, NRBC, NSE, S100B, GFAP, CPK-BB, IL-6, NPBI, UCHL-1. More sensitive and accurate instruments such as brain imaging (such as brain MRI), brain function (such as NIRS, aEEG), and long-term neuroassay should be used in the future to confirm biomarkers of neonatal brain damage.

Introduction

Biomarkers are compounds released from some organs that can be used to determine the physiological or pathological condition of that particular organ. Biomarkers can be extracted from blood, urine, cerebrospinal fluid (CSF) or any other body fluid in the first few hours of life. Biomarkers In infants with brain injury, may be able to detect the degree and place of injury. Recognizing the biomarkers of neonatal brain damage is an essential step in protecting the baby's nerves. Biomarkers are useful for brain damage screening, determine disease progression, identify damaged areas of the brain, and evaluate the effectiveness of neuroprotection techniques in clinical trials. Potential biomarkers must be highly validated because some of them are unrelated to specific organs, for example inflammatory mediators. Biomarkers are not commonly

used by doctors for caring brain-damaged babies. The article examines potential biomarkers that a clinical scientist may use to treat infants with hypoxic-ischemic encephalopathy.

Hypoxic-Ischemic Encephalopathy

Systemic asphyxia of brain appears as hypoxic-ischemic encephalopathy.[1] Systemic asphyxia happens approximately in 2% of full-term neonate and in 60% of very low birth weight or premature newborns.[2] About 20% to 50% of asphyxiated babies exhibit severe HIE die in the newborn period.[3] Up to 25% of the severe HIE survivors, have permanent neuropsychological problemsinclude: learning disabilities, cerebral palsy, with or without associated mental retardation, or epilepsy [4]. Systemic asphyxia (cause of HIE) may occur in three condition of delivery: prior (toxemia, placental abruption, maternal vascular disease, e.g.), during (difficult delivery, prolonged labor, abnormal presentation, etc.), or after (shock, sepsis, respiratory distress, etc.). Now, Diagnosis of hypoxic-ischemic injury is based on clinical Indicators. Asphyxia may have severe effects on many vitalorgans of neonates and can cause respiratory distress syndrome, necrosis of subcutaneous fat, disseminated intravascular coagulation, myocardial ischemia, metabolic complications, acute tubular necrosis, adrenal hemorrhage, neurological complications such as convulsion, brain paralysis and decrease in learning.[5,6] A physician's ability to predict the result of infants with HIE is not easy. The sarant grading system[7] exposes HIE based on clinical indicators. This grading system separates infants into three groups: severe categories, moderate or mild and this system measures the neurologic insult progress to estimate an infant's prognosis.[4] Amplitude integrated electroencephalogram (aEEG) is a new tool for bedside and can help exposing the intensity of damage and predicting prognosis.[8] Unfortunately, these two equipment are not as effective to predict

results of infants in hypothermia[9] and cannot provide data about the injury timing. MRI of the brain is useful to estimate the time of injury, however it is impossible to get an MRI in unstable infants.

Recently, hypothermia therapy has become the care's standard of HIE infants that can be moderate to severe. Before this treatment, infants underwent systemic supportive treatment without any specific treatment to prevent or improve persistent brain damage. Large multicenter randomized trials have shown hypothermia of HIE neonate with moderate to severe intensity is safe, showing progress in results, and has a combined number that must be treated.[9] Further step in protecting brain tissue is identifying biomarkers. This method can make clinical recommendation easy. Biomarkers can help physicians to identify infants that hypothermia is effective for them and others will require other new methods for protecting neurons. If clinicians can classify the neonates with biomarkers, they are not treated with useless and unnecessary methods. In addition, these neonates can be treated with better special treatments that are more compatible with biological characteristics in them. Biomarkers can be a base of future protective experiments for neurons and will lead the efficiency of intervention by short and long period.

Biomarkers of Hypoxic-Ischemic Encephalopathy

Until now, potential biomarkers (that exist in HIE infants) have been found. The biomarkers can be sampled of serum, CSF, saliva and urine. They contain biochemical markers such as lactate, LDH, NRBC count in the umbilical vein of newborns, neuron specific enolase (NSE), S100B, myelin basic protein, glial fibrillary acidic protein (GFAP), CPK-BB, umbilical cord Interleukin-6 (IL-6), pNF-Hand-nonprotein-bound iron (NPBI), ubiquitin carboxyl-terminal hydrolase L1 (UCHL-1).

As discussed above, the biomarkers' purpose is mainly to determine damage and

forecast long-period results. The least aggressive fluids are the best originals of biomarkers in very serious infants. Thus, a biomarker is produced ideally through saliva or urine. Biomarkers can be used after birth in ideal way for help estimating the time about onset of ischemic hypoxia injury and forecast the infants' results. Counter-intuitively, some biomarkers are not originating from brain and they can effectively predict some results such as long-term disability in neurodevelopment and death. IL-6 is one of these biomarkers produced by macrophages and T-cells. The concentrations of IL-6 was 5.5-fold more in comparison of the HIE neonates with none-HIE asphyxiated newborns and concentrations of IL-6 were majorly related to the intensity of HIE and the result of neurodevelopment at age of 2. Concentration of IL-6 maternal serum did not correlate with the risk of neonatal HIE.[10]

NRBC count in the infant's umbilical vein is considering to be a symptom of birth asphyxia.[11,12] In the blood circulation, NRBCs are not usually seen but in preterm infants on the third or fourth day of birth, little NRBCs' amounts can be recognized in the life's first week.[13, 14] In healthy newborns, count of NRBC is decreased after 12 hours of birth, and the amount of NRBCsis 20-30/m^3 after 48 hours. On the other side, issue hypoxia causes increasing loferythrocytes' and erythropoietin's amount. The NRBC's amount determines the erythropoietin's production. It can be concluded that stimulation of the fetal hematopoietic system mainly happens in the bone marrow by erythropoietin and then produces more RBC that increases in NRBC's count.[15-17] Its causes include: ABO or RH blood incompatibility, prematurity, more hematopoiesis followed by maternal diabetes, chronic diseases, fetal anemia, fetal anemia, intrauterine infections, preeclampsia, acute and fetal anemia or chronic asphyxia.[11,15,18]

S-100 is a major component of the cytosol in various types of cell as a calcium-binding protein. Specifically, there is a great concentration of S100B in glial cells. There are commercially available immunoassay kits that can recognize the S100B in many biological fluids (CSF, milk, urine, saliva, amniotic fluid, and blood).[19] In addition, infants and children have reference ranges in age three and there are reference ranges of urine's S100B for preterm and term healthy newborns. Concentrations of serum s100B have been reported for healthy children more than adults. Serum concentrations decreased in the first 6 months after birth. Also, the concentration of S100B protein in urine is greater in premature neonates toward term neonates and decreases continuously with the GA's progression.

In previous studies, there was a close relationship between biomarkers and HIE results, in one day after birth of infants with brain damage diagnosed by MRI, an increase in plasma brain-specific proteins including S100B in HIE newborns.[20] The results confirmed increased S100A8 expression was increased in all kinds of HIE infants and showed intensity of HIE.[21] Haptoglobin (HP) and S100A8 were up-regulated in HIE patients. HP is an antioxidant that removes free-hemoglobin from blood circulation and protects impairment of accordance due to free oxygen redicals.[15] In patients that are severely injured in spinal cord, presentation of HP is more.[22] Previously, several in-vitro and in-vivo studies revealed that HP in brain is up-regulated which is mediated by oligodendroytes.[23] Zhu et al., suggested HP as a definitive HIE's indicator, especially for early stage and mild HIE.[24]

Concentrations of S100B in the first urine were majorly greater in HIE patients toward control group after birth. This fact showed by Gazzolo et al., S100B was tested in samples of cord blood and was associated with HIE. Forty infants with HIE had great S100B protein concentration in cord blood compared with controls.[19] In the same study, S100B's density more than 2.02 μg/L had 86.7% sensitivity and 88% specificity f or estimating severe or moderate HIE' development.

Gazzolo et al., also showed that if concentration's cut-off of S100B is 0.41 µg/L, sensitivity and specificity will be 91.3% and 94.6% for estimating the HIE's development, respectively. Sensitivity and specificity can be respectively increased to 100 and 98.8% if urine samples were gathered at 4-72 hours after birth.[19] One hundred thirty two term-infants' study, S100B's concentrations were greater in neonates' urine who died or tolerated perinatal asphyxia and urine S100B above 1 µg/L forecasted death in neonates with 100% sensitivity and specificity. The same group's study showed that S100B's concentration in urine was not changed by renal failure.[25]

Glial fibrillary acidic protein (GFAP) is a cytoskeleton protein in astrocytes and is only observed as intermediate filament in the blood when host cell dies. GFAP have been associated with a little Cardiac output in adults after cardiac arrest, stroke or traumatic injury of brain.[6] GFAP has been utilized as an indicator of mortality or poor neurological results in kids needing extra corporeal membrane oxygenation.[12,13,26] A pilot study compared 23 HIE neonates who met the criteria for hypothermia with 23 NICU patients without neurologic injury. In first group they met the criteria for hypothermia and in second one they did not have neurologic injury.[5] The HIE patients had significantly raised concentrations of GFAP when they compared with second group (control ones). Also, GFAP more than 0.15 ng/mL (or eaqual to it) upon NICU admission we predicted an unusual MRI of brain.

After neonatal asphyxia, there are some other serum biomarkers to estimate long-period neurologic deficiencies. A recent meta-analysis[16], pooled data from published studies of neonatal HIE biomarkers that followed patients more than 12 months of age. Serum and CSF concentrations of IL-1b, IL-6, and serum NSE were predictive of abnormal outcomes. In addition, high GFAP concentrations in CSF were predictive of death.

Enolases expressed in all tissues as enzymes are capable of glycolysis, which neuron specific enolase belongs to this family. There are three subunits (γ, β and α) in this family and separate genes encoded each one of them. These subunits produce five kinds of isoenzymes: $\gamma\gamma$, $\beta\beta$, $\alpha\beta$, $\alpha\gamma$ and $\alpha\alpha$. Isoenzyme $\alpha\alpha$ (Enolase 1) is found in kidney, liver, adipose tissue and spleen. isoenzyme $\beta\beta$ (Enolase 3) is enolase for muscle. isoenzyme $\gamma\gamma$ (Enolase 2) is named NSE and found in neuroendocrine cells, peripheral and central neurons. We can detect kinds of cells with enolase isoenzymes: neurons and glia only respectively express NSE and enolase.[1] Platelets have the least quantities of this family (0.045% of platelets' soluble protein); although the major enolase of platelets is $\alpha\gamma$ subunits. Great NSE amounts in serum and CSF are associated with poor outcome of cardiac arrest,[27] cerebrovascular accident in patients[28] and traumatic brain injury in pediatrics.[13] NSE's diagnosis of serum is needed to happen after disruption of the blood-brain barrier or nerve death. In Animal's models[29] we observed a relation between the NSE's amounts and cortical injury's volume following by a traumatic injury of brain. Raised NSE's concentrations are associated with a poor prognosis in infants during cardiac surgery even if parallel samples of CSF do not show high NSE's levels.[30]

Celtik et al., prospected that indicator of HIE's severity can be serum neuron specific enolase[31]. Matching to ROC's curves, NSE in serum above 40 µg/L gained in period of 4 and 48 hours can help to recognize none or mild HIE neonates from moderate or severe HIE ones. Furthermore, NSE for prediction of poor outcomes with cut-off value 45.4 µg/L were found to be better than predictive values for severity degree of HIE.[31]

Analyzing brain's MRI in HIE patients have shown patterns that they are most common in injuries of brain including injury of basal ganglia, injury to watershed areas of the cortex and focal or diffuse cortical injury.

Two assessments about correlation between HIE biomarkers with various patterns found in MRI of brain injury showed that great concentrations of GFAP in the life's first 2 days in infants during hypothermia of whole body is related to unusual brain.[5,18,32] UCHL-1 (in cell bodies of neuron) and pNF-H1 (in white matter of brain regions) was measured in serum and compared between severe HIE patients and control group. There were correlations between serum amounts of these biomarkers and patterns in MRI's injury. These were pilot studies carried out on small numbers of patients so they require more validity. The capability of using biomarkers in combination with brain MRI improves the capability to forecast the outcome in patients with HIE. For instance, mixing biomarker's pathway include NSE with other diagnostic ways like MRI, makes the long-period prognostic prediction better.[13]

The last interesting group of potential biomarkers is neurotrophins. One of them is a binding neurotrophin to the p75NTR and TrkB receptors and named BDNF (Brain derived neurotrophic factor). This neurotrophin protects existing nerve's life and enhances the differentiation and growth for neurons and synapses which they are formed newly. A greater amount of BDNF is observed in cord plasma of HIE newborns when they compared with normal neonates.[17] The poor neurologic outcomes were predicted by great levels of BDNF. Documents has gained in Bejot's laboratory about increasing concentrations of brain BDNF after neonatal HIE in rodent subject and these reports are similarly to reports in the post-stroke found by others.[11] Migration of stem cells can be increased by BDNF. This fact is postulated by researchers.[33] From animal models, we could guess that the high concentration of BDNF in plasma reflects the great BDNF's concentration in the brain, which is released by nerve cells and astroglia cells and is trying to heal brain cells.

NPBI (Non-protein iron) was rated in all samples of blood. Elevated levels of NPBI expose proteins and lipid membranes to free radical attack, resulting in oxidative damage. The free iron's role was reported by many studies in oxidative damage of post-anoxic. Ciccoli et al., shows neonates in hypoxic condition release further iron in compare normal adults in normoxic condition.[34] Buonocore et al., reported that the NPBI's plasma amounts is greatly predictive in brain damage and intrauterine pain.[35] The NPBI's amounts were majorly greater in severe asphyxia neonates in pregnancy in six hours after birth, when levels of free iron in these neonates' plasma showed a declining tendency and were associated with hypothermia possibly. Therapeutic hypothermia is showed by multiple studies that not only reduces injury of brain and improves long-period results but also reduces the amounts in reactive oxygen species that are causing oxidative damage.[34,36]

Future Directions

One of the most hopeful therapies for protecting neuron is hypothermia and it has appeared in the last decade. This therapy will be baseline in future agents for protecting neuron. Although, 12.5% of infants respond suitably when they treat with hypothermia. Biomarkers can guide physician to detect infants who respond to non-hypothermia responders. Non-responding patients can be chosen for adding new neuroprotection methods. Biomarkers can estimate the injury's time. Hypoxic injury usually starts in the womb so because of the importance of time estimation, infants will not benefit from hypothermia if a lot of time have passed since the brain's injury. This may be an explanation of some HIE babies do not respond to hypothermia. In addition, Injury's time has major forensic consequences for the obstetrics and gynecology group caring for baby.

The use of biomarkers for neonatal brain damage allows more individual care of infants. For example, some babies could have greater components in inflammation toward other ones. We can treat these patients by

factors that make the cascade in inflammation less active. In addition, serum amounts of biomarkers can be used to observe baby's reaction to drugs. The decrease of plasma concentrations of biomarker can also represent endogenous tissue retention..

Discussion

It has been documented that most of acute and chronic condition could increase NRBC count through helping the erythropoietic activity. Absolute NRCB count in combination with HIE grade can be used for prognosis the risk of complications of asphyxia. Furthermore, it is found that NRBC count with more than 11 per 100 WBC, had sensitivity and specificity of 85% and 90%, respectively in estimating complications of asphyxia.[20] Previous studies have suggested NRBC as a hematopoietic marker in infants and its association with intrauterine hypoxia.[35,37,38] Serum and cerebrospinal fluid (CSF) interleukin-1b, serum-cell-neuron-specific interleukin-6 and cerebrospinal fluid (CSF), four markers potentially predict abnormal results. The nature of CSFs neurons is a potential predictor of poor outcomes. Serum interleukin 1b also showed higher level heterogeneity (30.6%). This is likely a reflection of outcome determination Oygür et al.,[39] and Aly et al.,[40] used less sophisticated outcome measures (clinicalexamination and the Denver Development Screening Tool) at a younger age of 12 months. The other two markers in the study, serum interleukin 6 and cerebrospinal fluid interleukin-1b, showed very little heterogeneity. However, those studies also demonstrated significant differences in outcome methods. Interestingly, both serum interleukin-6 and cord blood interleukin-6 are predictors of outcome, suggesting that interleukin-6 may also be predictive in the first few hours of life.[41] This suggests that interleukin-1b and interleukin-6 are potential biomarkers of brain damage and outcome. Animal studies justify the role of inflammatory markers in the pathogenesis of injury. Measurement of interleukin-1b and interleukin-6 mRNA and protein expression during recovery from hypoxic-ischemic injury in rodents increased significantly compared with controls.[42] Interleukin-1b was elevated 3, 6, and 10 hours after ischemia. The role of interleukin-1b is further supported by studies of transgenic mice deficient for interleukin-1b activation that exhibited reduced brain injury after a hypoxic-ischemic insult.[43] Selectively, this suggests interleukin-1b and interleukin-6, as potential candidate biomarkers, warrant further prospective experiments to confirm their use and characterize a neonatal brain. Human studies of cytokine levels and magnetic resonance spectroscopy show that elevated serum interleukin-1b and interleukin-6 levels are associated with increased lactate / choline ratios in deep gray nuclei, suggesting that cytokine levels are associated with brain injury.[41] Other biomarkers, such as the lactate / creatinine ratio, the first urine S100, and the interleukin-6 umbilical cord, are also prevalent, although they have only been identified in isolated studies. These three markers were measured in early infancy and are readily available. Urinary lactate / creatinine is difficult to interpret because lactate is a universal marker for anaerobic metabolism, and would not be brain-specific. Urine S100 is a very promising marker, and it was correlated with tissue injury in both porcine and sheep models of hypoxic-ischemic injury.[14,44] S100 is specific to the brain; only extra-neural tissue contains that of rat adipose tissue. The kidneys are excreted from the body and should be adjusted for serum creatinine in any prospective study.[45-47] Serum ionized Ca^{++} is a promising indicator, but its use is limited by the effects of pH and can only be measured and can be interpreted in physiological pH ranges between 7.7 and 4.7. This prevents its initial use in most encephalopathic infants, who are defined as pH < 7.1. NFkB activation in CD14 cells is a new concept, although the study was limited due to the small sample size and NFkB

activation. It is relatively difficult to measure because flow cytometry is not easily and quickly accessible in all centers.

Conclusion

Previous studies revealed that biomarkers such as brain-specific proteins (neuron-specific enolase (NSE), S100B, ubiquitincarboxy-terminal hydrolase-L1, total Tau) and cytokines, including interleukin (IL)-6, IL-1β, IL-10, IL-13,interferon-gamma, tumor necrosis factor alpha and brain-derived neurotrophic factor, are useful in diagnosing HIE and predicting nerve growth outcomes.[15,16,19] However, optimal sensitivity and specificity of these biomarkers have not been achieved, which has limited their clinical application.[48]

Further studies are needed to link and validate the clinical use of possible biomarkers of hypoxic-ischemic brain injury. More sensitive and accurate instruments such as brain imaging (such as brain MRI), brain function (such as NIRS, aEEG), and long-term neuroassay should be used in the future to confirm biomarkers of neonatal brain damage.

Conflict of Interests

Authors have no conflict of interests.

Acknowledgments

The author thanks the editors and the anonymous reviewers for insightful suggestions on this study.

References

1. Vannucci R. "Hypoxia ischemia: pathogenesis and neuropathology," in Neonatal perinatal medicine: diseases of the fetus and infant, 6th Edn, ed. Fanaroff A. (St. Louis, MO: Mosby), 1997: 856–891.

2. Low JA, Lindsay BG, Derrick EJ. Threshold of metabolic acidosis associated with newborn complications. Am J Obstet Gynecol 1997; 177(6): 1391-4.

3. MacDonald HM, Mulligan JC, Allen AC, Taylor PM. Neonatal asphyxia. I. Relationship of obstetric and neonatal complications to neonatal mortality in 38,405 consecutive deliveries. J Pediatr 1980; 96(5): 898-902.

4. Finer NN, Robertson CM, Richards RT, Pinnell LE, Peters KL. Hypoxic-ischemic encephalopathy in term neonates: perinatal factors and outcome. J Pediatr 1981; 98(1): 112-7

5. Martin RJ, Fanaroff AA, Walsh MC. Neonatal-perinatal medicine: diseases of the fetus and infant. St Louis: Elsevier/Mosby; 2011.

6. Ennen CS, Huisman TA, Savage WJ, Northington FJ, Jennings JM, Everett AD, et al. Glial fibrillary acidic protein as a biomarker for neonatal hypoxic-ischemic encephalopathy treated with whole-body cooling. Am J Obstet Gynecol 2011; 205(3): 251.e1-7.

7. Sarnat HB, Sarnat MS. Neonatal encephalopathy following fetal distress. A clinical and electroencephalographic study. Arch Neurol 1976; 33(10): 696-705.

8. Hellström-Westas L, Rosén I, 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(1): F34-8.

9. Thoresen M, Hellström-Westas L, Liu X, deVries LS. Effect of hypothermia on amplitude-integrated electroencephalogram in infants with asphyxia. Pediatrics 2010, 126(1): e131-e139.

10. Chiesa C, Pellegrini G, Panero A, De Luca T, Assumma M, Signore F, etal. Umbilical cord interleukin-6 levels are elevated in term neonates with perinatal asphyxia. Eur J Clin Invest 2003; 33(4): 352-8.

11. Bejot Y, Mossiat C, Giroud M, Prigent-Tessier A, Marie C. Circulating and brain BDNF levels in stroke rats. Relevance to clinical studies. PLoS One 2011; 6(12): e29405.

12. Vos PE, Lamers KJB, Hendriks JC,van Haaren M, BeemsT, Zimmerman C, etal. Glial and neuronal proteins in serum predict outcome

after severe traumatic brain injury. Neurology 2004; 62(8): 1303-10.

13. Berger RP, Adelson PD, Pierce MC, Dulani T, Cassidy LD, Kochanek PM. Serum neuron-specific enolase, S100B, and myelin basic protein concentrations after inflicted and noninflicted traumatic brain injury in children. J Neurosurg 2005; 103(1 Suppl): 61-8.

14. Kecskes Z, Dunster KR, Colditz PB. NSE and S100 after hypoxiain the newborn pig. Pediatr Res 2005; 58(5): 953-7.

15. Qian J, Zhou D, Wang YW. Umbilicalartery blood S100 betaprotein: atoolfortheearly identification of neonatalhypoxic-ischemicencephalopathy. Eur J Pediatr 2009; 168(1): 71-7.

16. Ramaswamy V, Horton J, Vandermeer B, Buscemi N, Miller S, Yager J. Systematic review of biomarkers of brain injury intermneonatal encephalopathy. Pediatr Neurol 2009; 40(3): 215-26.

17. Imam SS, Gad GI, Atef SH, Shawky MA. Cord blood brain derived neurotrophic factor: diagnostic and prognostic marker in fullterm newborns with perinatal asphyxia. Pak J Biol Sci 2009; 12(23): 1498-504.

18. Douglas-Escobar M, Yang C, Bennett J, Shuster J, Theriaque D, Leibovici A, et al. A pilot study of novel biomarkers in neonates with hypoxic-ischemic encephalopathy. Pediatr Res 2010; 68(6): 531-6.

19. Gazzolo D, Frigiola A, Bashir M, IskanderI, Mufeed H, Aboulgar H, et al. Diagnostic accuracy of S100B urinary testing at birth in full-term asphyxiated newborns to predict neonatal death. PLoS One 2009; 4(2): e4298.

20. Massaro AN, Wu YW, Bammler TK, Comstock B, Mathur A, McKinstry RC, et al. Plasma Biomarkers of Brain Injury in Neonatal Hypoxic-Ischemic Encephalopathy. J Pediatr 2018; 194: 67-75.

21. Yang L, Li D, Chen S. Hydrogen water reduces NSE, IL-6, and TNF-α levels in hypoxic-ischemic encephalopathy. Open Med (Wars) 2016; 11(1): 399-406.

22. Graham EM, Everett AD, Delpech JC, Northington FJ. Blood biomarkers for evaluationof perinatal encephalopathy: state of the art. Curr Opin Pediatr 2018; 30(2): 199-203.

23. Zhao X, Song S, Sun G, Zhang J, Strong R, Zhang L, et al. Cytoprotective role of haptoglobin in brain after experimental intracerebral hemorrhage. Acta Neurochir Suppl 2011; 111: 107-12.

24. Zhu Y, Yun Y, Jin M, Li G, Li H, Miao P, et al. Identification of novel biomarkers for neonatal hypoxic-ischemic encephalopathy using iTRAQ. Ital J Pediatr 2020; 46(1): 67.

25. Risso FM, Serpero LD, Zimmermann LJ, Gavilanes AW, Frulio R, Michetti F, et al. Perinatalasphyxia: kidneyfailure doesnot affect S100 Burinecon- centrations. Clin Chim Acta 2012; 413(1-2): 150-3.

26. Pelinka LE, Kroepfl A, Leixnering M, Buchinger W, Raabe A, Redl H. GFAP versus S100B in serum after traumatic brain injury: relationship to brain damage and outcome. J Neurotrauma 2004; 21(11): 1553-61.

27. Roine RO, SomerH, Kaste M, Viinikka L, Karonen SL. Neurological outcome after out-of-hospital cardiac arrest. Prediction by cerebrospinal fluid enzyme analysis. Arch Neurol 1989; 46(7): 753-6.

28. Hay E, Royds JA, Davies-Jones GA, Lewtas NA, Timperley WR, Taylor CB. Cerebrospinal fluid enolase in stroke. J Neurol Neurosurg Psychiatry 1984; 47(7): 724-9.

29. Costine BA, Quebeda-Clerkin PB, Dodge CP, Harris BT, Hillier SC, Duhaime AC. Neuron-specific enolase, but not S100B or myelin basic protein, increases in peripheral blood corresponding to lesion volume after cortical impact in piglets. J Neurotrauma 2012; 29(17): 2689-95.

30. Schmitt B, Bauersfeld U, Schmid ER, Tuchschmid P, Molinari L, Fanconi S, et al. Serum and CSF levels of neuron-specific enolase (NSE) in cardiac surgery with cardiopulmonary bypass: a marker of brain injury? Brain Dev 1998; 20(7): 536-9.

31. Celtik C, Acunaş B,Oner N, Pala O. Neuron-specific enolase as a marker of the severity and outcome of hypoxic ischemic encephalopathy. Brain Dev. 2004; 26(6): 398-402.

32. Utomo MT. Risk factors for birth asphyxia. Folia Medica Indonesiana 2011; 47(4): 211-4.

33. Borghesani PR, Peyrin JM, Klein R, Rubin J, Carter AR, Schwartz PM, et al. BDNF stimulates migration of cerebellar granule cells. Development 2002; 129(6): 1435-42.

34. Ciccoli L, Rossi V, Leoncini S, Signorini C, Paffetti P, Bracci R, et al. Iron release in erythrocytes and plasma non protein-bound

iron in hypoxic and non hypoxic newborns. Free Radic Res 2003; 37(1): 51-8.

35. Buonocore G, Perrone S, Longini M, Paffetti P, Vezzosi P, Gatti MG, et al. Non protein bound iron as early predictive marker of neonatal brain damage. Brain 2003; 126(Pt 5): 1224-30.

36. Hasegawa K, Ichiyama T, Isumi H, Nakata M, Sase M, Furukawa S. NF-kappaB activation in peripheral blood mononuclear cells in neonatal asphyxia. Clin Exp Immunol 2003; 132(2): 261-4.

37. Thilaganathan B, Athanasiou S, Ozmen S, Creighton S, Watson NR, Nicolaides KH. Umbilical cord blood erythroblast count as an index of intrauterine hypoxia. Arch Dis Child Fetal Neonatal Ed 1994; 70(3): F192-4.

38. Antonucci R, Porcella A PM. Perinatal asphyxia in the term newborn | Request PDF. J Pediatr Neonatal Individ Med 2014; 3(2): 1-14.

39. Oygür N, Sönmez Ö, Saka O, Yeğin O. Predictive value of plasma and cerebrospinal fluid tumour necrosis factor-α and interleukin-1β concentrations on outcome of full term infants with hypoxic-ischaemic encephalopathy. Arch Dis Child Fetal Neonatal Ed 1998; 79(3): F190-3.

40. Aly H, Khashaba MT, El-Ayouty M, El-Sayed O, Hasanein BM. IL-1β, IL-6 and TNF-α and outcomes of neonatal hypoxic ischemic encephalopathy. Brain Dev 2006; 28(3): 178-82.

41. Bartha AI, Foster-Barber A, Miller SP, Vigneron DB, Glidden D V., Barkovich AJ, et al. Neonatal encephalopathy: Association of cytokines with MR spectroscopy and outcome. Pediatr Res 2004; 56(6): 960-6.

42. Hagberg H, Gilland E, Bona E, Hanson LÅ, Hahn-Zoric M, Blennow M, et al. Enhanced expression of interleukin (IL)-1 and IL-6 messenger RNA and bioactive protein after hypoxia-ischemia in neonatal rats. Pediatr Res 1996; 40(4): 603-9.

43. Liu XH, Kwon D, Schielke GP, Yang GY, Silverstein FS, Barks JDE. Mice deficient in interleukin-1 converting enzyme are resistant to neonatal hypoxic-ischemic brain damage. J Cereb Blood Flow Metab 1999; 19(10): 1099-108.

44. Fujii EY, Kozuki M, Mu J, Ino Y, Ushioda N, Tomimatsu T, et al. Correlation of neuron-specific enolase and S100B with histological cerebral damage in fetal sheep after severe asphyxia. Brain Res 2004; 1018(1): 136-40.

45. Michetti F, Dell'Anna E, Tiberio G, Cocchia D. Immunochemical and immunocytochemical study of S-100 protein in rat adipocytes. Brain Res 1983; 262(2): 352-6.

46. Johnsson P, Lundqvist C, Lindgren A, Ferencz I, Alling C, Ståhl E. Cerebral complications after cardiac surgery assessed by S-100 and NSE levels in blood. J Cardiothorac Vasc Anesth 1995; 9(6): 694-9.

47. Michetti F, Gazzolo D. S100B protein in biological fluids: a tool for perinatal medicine. Clin Chem 2002; 48(12): 2097-104.

48. Douglas-Escobar M, Weiss MD. Biomarkers of hypoxic-ischemic encephalopathy in newborns. Front Neurol 2012; 3(144): 1-5.