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Comparsion of S100 and NSE Serum Level Concentrations as Primary Biochemical Indicators of Perinatal Asphyxia in Newborns

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ABSTRACT

Background: Neonatal hypoxic-ischemic encephalopathy (HIE) is a devastating condition resulting from a sustained lack of oxygen during birth. The aim of the present study was to evaluate serum concentrations of neuron-specific enolase (NSE) and the highly soluble glial protein S-100 as a marker of the severity of HIE.

Methods: We conducted a case-control study for 38 newborns diagnosed with moderate to severe hypoxic-ischemic encephalopathy and compared with 80 healthy controls. Two blood samples were taken to measure NSE and S100 levels, one at the birth time and the other in 24 h after birth for both groups. Comparison between cases and controls was performed using Fisher's exact or Chi-square test and Mann-Whitney U test. P value < 0.05 was considered statistically significant.

Results: In this study, the 118 neonates were studied in two groups, 38 neonates had asphyxia (case group) and 80 neonates were selected as the control group. The difference in S100 and NSE1 concentration was statistically significant between HIE cases and controls (P < 0.05) for the first taken sample.

Conclusion: levels of brain-specific proteins, such as S100, and NES can be assessed to identify infants at the highest risk of brain damage.

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1.INTRODUCTION

In recent years, the knowledge of early prognostic and diagnostic markers during the first days of life for babies who have sustained perinatal neurological insults such as perinatal asphyxia have gained rapidly. Perinatal asphyxia or hypoxic-ischemic encephalopathy (HIE) is a common cause of neonatal morbidity and mortality in the neonatal intensive care unit and of long-term neurologic disabilities among survivors (1, 2). The reported incidence of birth asphyxia in overall ranges from 0.1% to 0.5% in births, and 23% cause of neonatal mortalitAy (1). Asphyxia results from compromised placental or pulmonary gas exchange. This disorder can lead to hypoxia and hypercarbia. Asphyxia can occur before, during, or after birth. A variety of maternal, obstetric, and neonatal conditions predispose the fetus and newborn to asphyxia. These risk factors are associated with reduced blood flow and/or oxygenation to the tissues (3-5).

Hypoxic ischemic encephalopathy after perinatal asphyxia is a condition in which cerebrospinal fluid (CSF) and/or serum concentrations of brain-specific biochemical markers may be elevated (6-8). Of those brain-specific proteins, the brain-derived creatinine kinase (CK-BB), the highly soluble glial protein S-100, glial fibrillary acidic protein (GFAp), neurofilament protein (NFp) and the neuron-specific enolase (NSE) have been found to be released in high concentrations into the CSF of asphyxiated infants and correlated significantly with other indicators of long-term prognosis and neurological impairment at 1 year of age or at death (7).

The neuron-specific enolase is a dimeric glycolytic enzyme, containing two gamma subunits, originates predominantly from the cytoplasm of neurons and neuroendocrine cells. It is soluble and stable in biological fluids and its determination is not affected by hyperbilirubinemia or lipemia. Furthermore, it does not elicit immunological cross-reactivity with non-neuronal enolase (3, 7, 9, 10). Studies have shown associations between the severity of the neuronal injury and the concentration of S100, which has led to a special interest in S100 in asphyxiated newborns (11-14).

However, there have only been a few studies investigating brain injury biomarkers from blood sampled at birth. We could thus perform a retrospective case-control study of cord S100 concentrations taken from newborns and compare them with healthy controls. The aim of the study was to compare cord S100 and NSE concentrations between neonates with HIE and healthy controls. We hypothesized that HIE would correspond to higher cord S100 and NSE concentrations.

MATERIAL AND METHODS

Patients and controls descriptions

A total of 118 term newborn infants were selected from a university hospital (Shahid Akbarabadi) of Iran University of Medical Sciences in Tehran, Iran. The study was approved by the Ethical Committee of "Iran University of Medical Sciences, Faculty of Medicine". The study was started from 20 March 2016 to 20 March 2018.

All control infants had an Apgar score of 9 at 1, 5, and 10 minutes and were born 37/75+0.52 weeks of gestation after an uncomplicated delivery, with a mean umbilical artery acidosis pH of 7.02±0.14 in cases. Diagnosis codes for neonates suffering from HIE were identified using Sarnat (15). Clinical data were retrieved from the local database in HIE cases. The data consist of blood pCO2, base excess (BE) and actual bicarbonate (HCO3-) and PH. Data about pregnancy progress, birth, and the perinatal outcome were retrieved from the local obstetric database for both cases and controls. Informed consent was obtained for all parents of neonates before the collection of blood samples.

The diagnosis of asphyxia was made based on clinical signs, Apgar score at birth and acid-base(ABG) status.

The following inclusion criteria were used (all necessary): term newborn (36 completed gestational weeks), Apgar score < 7 at 5 minutes or other clinical signs of perinatal asphyxia, together with umbilical artery acidosis (pH < 7.10 and/or base deficit > 12 mmol/L), and clinical signs of asphyxia necessitating transfer to the NICU. Neonates with severe infection (sepsis or pneumonia) or severe malformations were excluded. All infants were neurologically examined daily during the first week of life and were classified with mild, moderate, or severe HIE.

NSE and S100 levels measurements

Two blood samples were taken to measure NSE and S100 levels, one in birth time and the other, between 24 h after birth. The blood samples were taken at similar time periods from all groups. All blood samples were immediately frozen (-20 C). Serum NSE was measured using ELISA (Zell Bio, Germany) and S100 protein quantified using ELISA (BioVendor, Czech Republic) according to the manufacturer's instructions.

Statistical analysis

The descriptive statistics were extracted with absolute or frequency, mean and standard deviation. The Kolmogorov-Smirnov test was used to evaluate whether the distribution of data was Gaussian. Comparison between proportions was performed using Fisher's exact or Chi-square test. Comparison of case and control in continuous parameters between groups was performed by the Mann-Whitney U test. Statistics were performed with aid of SPSS version 22.0 (IBM, Chicago, IL, USA) computer software for statistical computation. A two-tailed P-value < 0.05 was considered statistically significant.

RESULTS

In the present survey, the 118 neonates were studied. 38 neonates had asphyxia (case group) and 80 neonates were selected as the control group. Clinical findings, neonatal outcomes and laboratory parameters of all the studied infants are shown in Table 1. There were no differences between patient and control groups in terms of gestational age, birth weight, gender, height, head and maternal age (P>0.05 for all). Almost mothers in the control group were CS (P= 0.01). The first and fifth minute Apgar scores of cases were significantly lower than those in the control group (P> 0.001).

Table 1: Characteristics of the case and control group

Variable	Case (n=38)	Control (n=80)	P. value
Birth weight (g)	2961.84±115.23	3135.57±53.89	0.12
Maternal age	28.97±1.12	30.32± 0.64	0.26
Gestational age (week)	37.63± 0.58	37.75± 0.52	0.89
Gender (M/F)	25.13	38.40	0.08
Mode of delivery(CS/NVD)	22.16	62.18	0.01
Apgar score (1 min)	3.23± 0.32	8.92±0.05	<0.001
Apgar score (5 min)	5.73± 0.35	10±0	<0.001
Height	49.10± 0.77	50.43± 0.30	0.05
Head	34.1± 0.52	34.53± 0.16	0.29
Hco3	16.05±6.15	-	-
Be	-13.12±9.80	-	-
Pco2	65.82±24.23	-	-
PH	7.02±0.14	-	-

There were significant differences between case and control in terms of serum NSE and S100 concentrations of the first blood samples. (p=0.02 and 0.0001). Serum concentrations of NSE at different sampling times (NSE1, NSE2, and S1001, S1002) in the case groups and the control group are shown in Table 2 and 3. No significant difference was detected in serum NSE and S100 levels between cases and the control group in the second samples. The mean serum concentrations of the second samples decreased in two studied groups, however, they were not significantly higher in the first samples compared with the second samples (P>0.05).

Table 2: Serum concentrations of NSE at different sampling times (NSE1, NSE2) in the patient group and the control group

Group	NSE_1 (µg/l)	P. value	NSE_2 (µg/l)	P. value
Case	11.20± 1.61	0.02	11.1± 1.82	0.49
Control	16.35± 1.32		12.86± 1.25	
Total	14.74±1.06		12.47± 1.05	

Table 3: Serum concentrations of S100 at different sampling times ($S100_1$, $S100_2$) in the patient group and the control group

Group	$S100_1$ (µg/l)	P. value	$S100_2$ (µg/l)	P. value
Case	162.38± 25.70	0.0001	102.66± 52.21	0.68
Control	37.38± 16.8		84.22± 19.33	
Total	76.17± 15.01		88.34±18.88	

DISCUSSION

Prenatal hypoxic-ischemic encephalopathy (HIE) is mainly caused by a combination of hypoxia and ischemia due to a decreased oxygen and blood follow to the brain of newborns. One of the common complication affecting newborns is HIE and that is the biggest cause of mortality among them.

Many cellular and extra-cellular processes are activated in the brain following hypoxic-ischemic injury. A number of molecules have been reported to be the potential biomarkers of HIE. Serum inflammatory proteins are readily measurable and may be useful biomarkers of injury phases(9). The information about specific regional brain injury as well as the presence of biomarkers of brain injury can be useful for understanding cellular mechanisms of the disease(16). The severity of brain injury and the knowledge of the anatomical pattern is important. By combining biomedical risk reduction and neuroprotective strategies, progress can be made in optimizing child's neurodevelopment (17).

The specific role of each of these important informative factors in brain injury is difficult to understand in many cases. One of the most common acute symptoms that precede with prenatal hypoxemia-ischemia is choking. In addition to the biochemical changes, hypercapnia can occur and cause circulatory effects(18).

Due to hypoxia, ischemia, cerebral hypercapnia, and acidosis, the production of glycolysis and lactic acid is increased at the biochemical level. It also decreases the synthesis of high-energy phosphate compounds, induces extracellular potassium and intracellular calcium accumulation, produces harmful free radicals that can damage plasma membrane of the cells and alter the metabolism of neurotransmitters and excitatory amino acids. This cascade of events leads to hypoxic-ischemic injury and neuronal death(19).

A high percentage of neonates with HIE suffer from tissue damage. Redistribution of the blood flow to organs such as the brain may lead to the development of this complication. Recent surveys have shown that severe brain damage stimulates the release of TNF-a and inflammatory molecules, including Intercellular Adhesion Molecule 1 (ICAM-1), and interleukins into the systemic circulation. Certain attacks on the brain are followed by increased permeability of the blood-brain barrier, which is very important in maintaining brain hemostasis and supports the hypothesis that the tissue damage is the consequence of the release of inflammatory molecules(20).

In the present study, we found a decrease in the serum neuron-specific enolase level (NES) in patients with perinatal asphyxia. Enolase acts as a marker of acute brain damage. Our study, despite the small number of patients, revealed that the NSE level in the blood of neonates may be correlated with the severity of encephalopathy and brain injury.

Outside of the neonatal period, the NSE has been studied as a marker of brain damage in various neurological diseases in adults and children. The NSE levels in both the plasma and CSF have been shown to be strongly correlated with the extent of neurological damage. Several findings have been reported in experimental studies demonstrated that the NSE concentrations in the CSF of 49 neonates with HIE at 12 hours and 72 hours after birth were correlated with both the severity of encephalopathy and outcome (death or cerebral palsy) Garcia-Alix et al(7). In another study, Thornberg et al. (21) analyzed the NSE levels in the blood and CSF of asphyxiated neonates and found no correlation between the NSE level in the CSF and brain damage; however, the infants with the highest NSE levels died.

Elevated serum S100B levels have been found in our patients. This finding is in agreement with the results of other studies, including Gazzolo et al. (13) and Qian et al. (22) These previous studies reported that HIE patients have significantly high S100 concentrations in both urine and cord blood after birth compared with controls. S100B is a protein that plays a trophic role during nervous system development (23). However, it's over expression can have deleterious effects. A high S100B concentration results in potent activation of inducible NO synthase and subsequent generation of NO and astrocyte cell death (24).

CONCLUSION

Taken together in the present survey we investigated the serum level of S100 and NES level in HIE affected neonates. A combination of very early indicators, such as acid-base values, the clinical condition of the infant, the Apgar score, the presence of HIE, early electroencephalography (EEG)/amplitude integrated electroencephalography (aEEG)s findings, and the levels of brain-specific proteins, such as S100, and NES may be assessed to identify infants at the highest risk of brain damage.

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CONFLICT OF INTEREST

None

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