

STUDY OF NEURON SPECIFIC ENOLASE (NSE) IN PERINATAL ASPHYXIA & ITS ROLE AS AN EARLY MARKER OF BRAIN INJURY

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ABSTRACT

BACKGROUND

Perinatal asphyxia is a major cause of neurological morbidity in infants. Hypoxic ischaemic encephalopathy (HIE) after perinatal asphyxia is a condition in which serum concentration of Neuron specific enolase may be elevated. There are very few studies about its usefulness in asphyxiated newborns.

AIMS AND OBJECTIVES

To determine the serum levels of Neuron Specific Enolase (NSE) in newborns with perinatal asphyxia and its relation with different stages of hypoxic ischaemic encephalopathy.

METHODS

We have determined the serum levels of NSE by ELISA method in 100 asphyxiated newborns and 100 healthy newborns (control group). Blood samples were taken on day 1 and day 3 of life in all newborns.

RESULTS AND CONCLUSION

The mean serum values of NSE were found to be decreased on day 3 in asphyxiated neonates and negative correlation was seen between day 1 and day 3 for NSE.

KEYWORDS

Hypoxic Ischaemic Encephalopathy (HIE), Neuron Specific-Enolase (NSE), Perinatal Asphyxia.

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INTRODUCTION: Hypoxic-ischaemic cerebral injury that occurs during neonatal period is one of the most commonly recognised causes of severe, long term neurological deficit in children. Perinatal asphyxia is a common cause of neonatal morbidity and mortality in neonatal period and long term neurologic disabilities among survivors.⁽¹⁾ Hypoxic-ischaemic encephalopathy (HIE) of the newborn occurs with the incidence of 1-4/1000.⁽²⁾ Between 20% and 50% of newborn infants affected by perinatal brain injury die during the newborn period, and 25-60% of the survivors suffer from permanent neurodevelopmental handicaps, including cerebral palsy, seizures, mental retardation, and learning disabilities.^(3,4) Various indicators of brain damage have been investigated in the last decade.⁽⁵⁻¹¹⁾ Early recognition of HIE is important in guiding the management of these neonates and justifying administration of certain drugs.

Some authors have suggested that biochemical indicators may be more effective than results of clinical evaluation, pH in cord blood, Apgar score, electroencephalographic & neuroimaging data. The biochemical monitoring should aim at measuring the routine parameters as well as recent specific biomarkers of brain injury to assess regional brain damage after perinatal asphyxia in neonates. Among the biochemical markers of brain injury, NSE was studied in details in different stages of HIE. Neuron-Specific Enolase (NSE), a homodimer of the gamma form of enolase, is localised in the cytoplasm of neurons and cells of neuronal origin.

NSE is an enzyme which catalyses the conversion of 2-phosphoglycerate to phosphoenolpyruvate in the glycolytic pathway, and also the reverse reaction in gluconeogenesis.⁽¹²⁾ Since neurons require a great deal of energy they are very rich in glycolytic enzymes such as NSE. Physiologically, NSE is present only in negligible amounts in the peripheral blood. CSF- NSE concentration is 17.3±4.6 ng/mL; serum concentration is 8.7±3.9 ng/mL.⁽¹³⁾ Its presence in cerebrospinal fluid and blood is attributed to cell destruction. Serum NSE level is a marker for neuronal damage after traumatic brain injury, tumour, and neurodegenerative diseases.⁽¹⁴⁾

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In the present study, we investigated the serum values NSE in asphyxiated newborns and their relation with different stages of HIE.

METHODOLOGY: The study was undertaken with the approval of Institutional Ethical Committee of the medical faculty of S.A.I.M.S. Medical College and PG Institute, Indore, MP. The study included 100 asphyxiated newborns as the study group and 100 healthy newborns as control group.

Inclusion Criteria: The newborns admitted in the Department of Paediatrics and its neonatal unit was enrolled for the present study. Gestational age, birth weight, relevant perinatal history, findings on physical examination and systemic signs were recorded on a predesigned pretested proforma in both the groups. The study group was further divided according to Sarnat and Sarnat classification as: No HIE group, mild HIE (grade I), moderate HIE (grade II) and severe HIE (grade III).

Exclusion Criteria: Predefined exclusion criteria for both the groups were congenital anomalies, tumours, maternal drug addiction, severe infections and congenital mental disorders.

Blood Sampling and Analysis: Blood samples (1-2 mL) were collected on day 1 and day 3 of life. Serum was carefully separated by centrifugation and then stored in aliquots at -70°C until analysis. Method-Immuno-enzymatic colorimetric method for quantitative determination of NSE concentration in human serum.

Principle: The NSE ELISA test is based on simultaneous binding of human Neuron Specific Enolase by two monoclonal antibodies, one immobilised on Microwell plates and other conjugates with horseradish peroxidase (HRP). After incubation the bound/free separation is performed by a simple solid-phase washing, and then the TMB-Substrate solution (TMB) is added.

After an appropriate time has elapsed for maximum colour development, the enzyme reaction is stopped and the absorbance is determined. The hNSE concentration in the sample is calculated based on a series of calibrator. The colour intensity is proportional to the hNSE concentration in the sample.

Reagents and Materials: Reagents and materials supplied in the kit:

1. Calibrators (CAL 0-CAL5), 2 vials each calibrator, lyophilised.
2. Controls, 2 vials each, lyophilised negative and positive control.
3. Incubation Buffer, 1 vial, 50 mL, phosphate buffer (50 mM) pH 7.4; BSA(1 g/L).
4. Conjugate, 1 vial, 1 mL, monoclonal anti-hNSE antibody conjugated with horseradish peroxidase (HRP).
5. Coated Microplate, 1 breakable microplate, monoclonal anti-hNSE antibody adsorbed on the microplate.
6. TMB Substrate, 1 vial 50 mL, H₂O₂-TMB (0.26 g/L), (avoid any skin contact).
7. Stop Solution, 1 vial 15 mL, Sulphuric acid (0.15 mol/L), (avoid any skin contact).
8. Wash solution 50X concentrate, 1 vial, 20 mL NaCl (45 g/L); Tween 20 (55 g/L).

Procedure: Allow all reagents to reach room temperature, (22°C-28°C). Unused coated Microwell Strips should be released securely in the file pouch containing desiccant and stored at 2°C-8°C. To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials. As it is necessary to perform the determination in duplicate in order to improve accuracy of the test results, prepare two wells for each point of the calibration curve (CAL 0-CAL 4), two for each control, two for each sample, and one for Blank.

Reagent	Calibrator	Sample/Control	Blank
Calibrator CAL 0-CAL-4	25 µL		
Sample/controls		25 µL	
Diluted conjugate	100 µL	100 µL	
Incubate at room temperature (22°C - 28° C) for 1 hour. Remove the contents from each well and wash the wells 3 times with 300 µL of diluted Wash solution.			
TMB Substrate	100 µL	100 µL	100 µL
Incubate at room temperature (22°C - 28°C) for 15 minutes in the dark.			
Stop Solution	100 µL	100 µL	100 µL
Shake the microplate gently, Read the absorbance (E) at 450 nm against Blank within 5 minutes.			

Calculation of Results:

1. The concentration of (X) of each standard was plotted against its absorbance (Y) on a graph paper.
2. The NSE value of patient was obtained by reference to the standard curve.

Reference Values: The serum values are comprised in the following intervals:

	hNSE
Normal range	0-12 ng/mL
Pathological value	>12 ng/mL

STATISTICAL ANALYSIS: Descriptive statistics and analytical tests were performed using SPSS software version 16. Data were presented as Mean±SD or number (%). A p-value of <0.05 was considered statistically significant. Unpaired t test & paired t test used for comparison between cases & control data. Correlation was calculated by Pearson correlation coefficient (2- tailed).

RESULTS: Total 100 asphyxiated newborns and 100 healthy newborns were included in study. The mean gestational age (weeks) of cases is 38.02±2.53 and of controls is 38.44±2.22. The mean birth weight (kg) in cases and controls were 2.68±0.69 and 2.77±0.54 respectively. Out 100 asphyxiated newborns, 18 had no HIE, 20 developed HIE grade I, 41 grade II, and 21 grade III.

The mean values for NSE in control and cases on Day 1 are 12.15±10.23 and 49.78±39.43 respectively, while mean values for NSE in control and cases on Day 3 are 8.25±8.55 and 35.71±31.71 respectively (Table 1). The mean value of serum NSE were statistically highly significant in the asphyxia group compared with the control group on Day 1 and Day 3 (p <0.001) (Table 1). The mean values of NSE were significantly decreased on Day 3 as compared to Day 1 in cases (p <0.001) A negative correlation was observed between Day 1 and Day 3 for NSE (Table 2). Among the infants in whom HIE developed, first day serum NSE levels were 25.44±18.40 ng/mL in those with stage I, 49.53±19.90 ng/mL with stage II and 97.80±47.07 ng/mL with stage III (Table 3). There was also a significant association between the serum level of the first day NSE and severity of HIE. Serum NSE was higher in infants with moderate or severe HIE compared with infants with no or mild HIE on day 1 and on day 3. We found that in the whole group of asphyxiated neonates, as well as within the subgroup with or without HIE, there was a significant decline from day 1 and day 3 of life (p <0.001) in NSE levels.

NSE (ng/mL)	Control	Mean±SD Control	Cases	Mean±SD Cases	P Value
Day 1	100	12.15±10.23	100	49.78±39.43	<.001
Day 3	100	8.25±8.55	98	35.71±31.71	<.001

Table 1: Mean Values of NSE on Day 1 and Day 3 in Control and Cases Group and Their Comparison

NSE (ng/mL)	Cases	Mean±SD	r value	P value
Day 1	100	49.78±39.43	- 0.942**	<.001
Day 3	98	35.71±31.71		

Table 2: Comparison of Mean Values of NSE on Day 1 and Day 3 in Cases of Birth Asphyxia and Their Correlation

Stages of HIE	Day 1 Mean±SD	Day 3 Mean±SD	r value	P value
No HIE (0) (n=18)	21.01±23.17	14.44±20.10	- 0.988**	<.001
I (n=20)	25.44±18.40	20.24±19.08	- 0.698**	<.001
II (n ₁ =41) (n ₃ =40)	49.53±19.90	35.24±17.86	- 0.954**	<.001
III (n ₁ =21) (n ₃ =20)	97.80±47.07	71.51±41.35	- 0.911**	<.001

Table 3: Comparison of Mean Values of NSE on Day 1 and Day 3 in Different Stages of HIE and Their Correlation

DISCUSSION: In adults, neuronal necrosis and apoptosis after global ischaemia are slow and last for several hours to several days. Studies in perinatal animals are suggestive of quicker cellular destruction. Energy substrates continue to run down for 12 to 48 h. after hypoxia in the neonatal brain. As neuroprotective interventions may be harmful, it is important to find early and reliable indicators of brain damage or of poor long term prognosis to initiate or end neuroprotective treatment. Cranial tomography, somatosensory evoked potentials, and magnetic resonance tomography are useful for prognosis, but not in the first 24 hours after birth. Magnetic resonance spectroscopy reveals brain energy compromise, but is not practicable in most clinical situations.

EEG is a useful diagnostic tool for assessing encephalopathy but interpretation can be difficult especially if paralytic or sedative agents had been used earlier. Therefore, reliable and accurate markers are highly awaited. NSE is such a promising biomarker for severity of brain injury and prognosis following asphyxia. In the present study, we have determined serum level of NSE in asphyxiated and healthy neonates. Serum NSE concentrations in the first day of life were significantly elevated in cases compared with the healthy controls, and these elevated concentrations were associated with the severity of asphyxia. In our study, NSE concentrations decreased on day 3 as compared to day 1.

Neonate with severe asphyxia at any time had significantly more elevated mean serum levels of NSE compared to the group with mild asphyxia and to the control group. The values of control group were also significantly lower in comparison with that of mild asphyxia. In neonates with severe asphyxia, NSE values decreased constantly from day 1 to the third day of life. Our results are in concordance with Giuseppe, Distefano et al.⁽¹⁵⁾ Also Verdu Perez et al found that the presence of elevated NSE values in blood after perinatal asphyxia can be sensitive indicator of brain damage.⁽¹⁶⁾ In a similar study by E Thonbergh, increased values of NSE was significantly increased in CSF of infants with HIE than control infants.⁽¹⁷⁾

CONCLUSION: The mean value of NSE was decreased in different stages of HIE on day 3 as compared to day 1 and a negative correlation was observed between day 1 and day 3 for NSE in no HIE, HIE I, HIE II and HIE III stages. Presence of NSE is attributed to neuronal cell destruction due to hypoxia. It is believed that NSE is released in blood by tissue damage where it has a half-life of around 48 hours. The serum NSE concentrations increased considerably after birth asphyxia, and these increases were associated with the severity of encephalopathy and a poorer outcome. Hence, NSE might have an important role following injury to the CNS, and serum concentrations appear to be a good predictor of outcome in HIE. However, more investigations are required for better understanding of the role of this cytokine in cerebral injury caused by hypoxic insult.

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