PROGNOSTIC VALUE OF NEURON SPECIFIC ENOLASE IN SERUM FOR OUTCOME OF INFANTS WITH BIRTH ASPHYXIA

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ABSTRACT

Background: Birth asphyxia [BA] and the resultant hypoxic ischemic encephalopathy [HIE] is a common cause of neonatal morbidity and mortality and neurologic disabilities among survivors .It is important to find an early and reliable indicator of brain damage and of poor long-term prognosis to initiate or end neuroprotective treatment.

Objective: Assess if neuron specific enolase [NSE] can be used as a serum biochemical marker of brain damage in neonates exposed to perinatal asphyxia [PA], and if it can be used in predicting long- term outcome in these infants.

Study design: This prospective study was carried out on 50 neonates who were delivered in the Obstetric Department of Zagazig University Hospitals and admitted to the neonatal intensive care unit [NICU]. The patient group comprised 30 full term neonates who developed symptoms and signs of HIE. Twenty healthy full term neonates of matched age, sex and weight served as a control group. All neonates were subjected to; history-taking, clinical examination and laboratory investigations, including measurement of serum NSE by enzyme immuometric assay. In addition survivor neonates were subjected to follow-up at 6 month and 12 month.

Results: Apgar scores and arterial cord blood pH were significantly lower in HIE neonates than that in control neonates. Meanwhile, serum NSE and arterial cord blood base deficit were significantly higher in HIE neonates than that in control neonates. Serum NSE was significantly higher in neonates suffering HIE grade II & grade III than that in HIE grade I& control neonates. Furthermore, serum NSE was very highly significantly increased in neonates who had neurological sequalae than that in control neonates.

Conclusion and recommendation: Serum NSE correlated significantly with HIE grades II&III and neonates who suffered neurological sequalae. So it may be used as a marker of grades II& III and neurological sequalae in asphyxiated neonates. However, it can not be used to prevent BA.

INTRODUCTION

Birth asphyxia [BA] is a condition where there is an impaired gas exchange leading to hypoxemia, hypercapnea and acidosis in fetus or neonate. The frequency of BA is 1-1.5% live births. An infant suffering severe perinatal asphyxia usually has poor color (cyanosis), perfusion, responsiveness, muscle tone, and respiratory effort, as reflected in a low 5-minute Apgar score [1] . Hypoxic ischemic encephalopathy develops in 33% of asphyxiated neonates [2] .

Early recognition of HIE is critical in guiding the management of these neonates in an attempt to determine the cerebral injury and to predict the neurologic outcome. Various investigations have been used as an adjunct to the clinical examination [3] .

Several biochemical markers [brain-specific creatine kinase, lactate/creatinine proportion, glialfibrillary acidic protein, uric acid, hypoxanthine, NSE and protein S in the blood or cerebro-spinal fluid] have been examined in neonates with PA [4] .

Neuron specific enolase belongs to the family of enolase enzymes, present in all tissues and organisms capable of glycolysis. NSE is soluble and stable in biological fluids and its measurement is not influenced by hyperbilirubinemia or lipemia. Furthermore, it doesn't elicit immunological cross-reactivity with non-neuronal enolase [5] . So this work aimed to study the circulating serum NSE in neonates with HIE because of PA. Furthermore, assessment of NSE as an indicator of short-and long term neurologic sequalae in these infants.

SUBJECTS AND METHODS

This prospective study was carried out on 50 neonates who were delivered in the Obstetric Department of Zagazig University Hospitals and admitted to the NICU, during the years 2014 and 2015.

The patients were divided in to two groups:

Group [I]: Included 30 full term neonates who developed symptoms and signs of HIE, as indicated by Sarnat and Sarnat [6] .

The selection criteria for HIE included [at least two]:

1. Evidence of fetal distress [abnormal fetal heart rate patterns and/or meconium-stained amniotic fluid].
2. Apgar score of 0-3 at 5- minute, improving to ≥ 7 by 10- minutes [1] .
3. Evidence of neonatal respiratory distress.
4. Umbilical cord arterial pH of <7.2 with base deficit of >10 m mol/L.
5. Abnormal neurological symptoms and signs, denoting mild HIE [HIE I], or moderate HIE [HIE II] or severe HIE [HIE III] [6] .

- Group [II] : Twenty full-term neonates of matched age, sex and weight with normal physical finding severed as a control group .They satisfied the following criteria:

1. Arterial cord blood pH of >7.2.
2. An Apgar score at 5 minutes of ≥7.
3. No maternal sickness.

Exclusion criteria for both groups were:

- Preterm infants [of < 37 weeks gestation].
- Central nervous system and cardiac malformations.
- Intrauterine infection.
- Congenital metabolic disorders.
- Maternal drug addiction.

All neonates were subjected to the following:

1. History-taking and clinical examination including; Apgar scoring, gestational age and neuromuscular development as indicated by Ballard scoring system [7] , anthropometric measurements, chest, heart, abdominal and neurologic examination.
2. Laboratory investigations

Cord blood samples were collected within 48 hours after birth and analyzed for:

A. CBC, using coulter counter.
B. Sepsis screen, including CRP, immature/total neutrophils and blood culture, using nutrient media, then subculture on suitable sugar media.
C. Liver and kidney function tests.
D. Arterial cord blood pH and base deficit.
E. Neuron-specific enolase level: recognized by enzyme immunometric assay utilizing enzyme immunometric assay Kit [Product No 420-10, September 1, 2012]. This kit is intended for the quantitative determination of NSE in human serum.

3. Follow up:

After 6 and 12 months, survivors were subjected to:

A. General physical examination.
B. Neurodevelopmental examination, according to **Thompson et al**[8]

4. Statistical Analysis was carried out using [SPSS=Release 12] for windows.

RESULTS

The results of this study are presented in tables [I-IV]

Table [I]: Demographic characteristics of HIE neonates [Group I] versus control neonates [Group II]:

Characteristics [S]	Group I [n=30]	Group II [n=20]	P-value
Gestational age [weak],X±SD	37.9±1.4	38.7±1.8	0.085
Birth weight [gram], X±SD	2951.8±575.5	3150.5 ± 416.4	0.072
Gender, n&[%]			
Boys	16 [53.3]	11 [55]	0.419
Girls	14 [46.7]	9 [45]	
Delivery route, n &[%]			
Vaginal	13 [43-3]	12 [60]	<0.05*
C/S	17 [56.7]	8 [40]	
Apgar score, X±SD			
1-minute	3.5±1.7	6.9±0.8	<0.01*
5-minute	5.1±1.5	8±0.7	<0.01*
Arterial cord blood pH, X±SD	6.77±0.65	7.25±0.84	<0.05*
Arterial cord blood base deficit [m mol/L], X±SD	16.5±3.12	5.3±2.03	<0.01*
NSE [µg/L], X±SD	41.8±17.3	15.8±6.4	<0.003*
Outcome, n&[%]			<0.004*
alive	27[90]	20[100]	
dead	3[10]	0[0]	<0.05*

C/S: cesarean section ***:** Significant **m mol/L:** millimolecule/liter **µg/L:** microgram/ liter **NSE:** neuron-specific enolase

X ± SD: mean± standard deviation **n:** number **%:** percentage

Table [II]: Correlation between serum NSE and each of gestational age, body weight, Apgar scores umbilical artery pH, and umbilical artery base deficit among asphyxiated neonates

Variable	Correlation coefficient [r]	P-value
Gestational age[week]	**0.071**	**0.708**
Body weight[grams]	**-0.051**	**0.787**
Apgar score[1- minute]	**-0.640**	**<0.001***
Apgar score[5-Minute]	**-0.741**	**<0.001***
Umbilical artery pH	**0.181**	**0.337**
Umbilical artery base deficit [m mol/L]	**0.693**	**<0.001***

***:** significant **NSE:** neuron specific enolase **M mol/L:** millimolecule/liter

Table[III]: Clinical and biochemical characteristics in neonates with different stages of hypoxic-ischemic encephalopathy [HIE], and those With neurological sequels, presented as mean± standard deviation [X ±SD],compared with control group.

Parameter	Control [n=20]	HIE Stage I [n=9]	HIE Stage II [n=13]	HIE Stage III [n=8]	Neurological Sequels [n=7]
Gestational age [week]	38.7±1.8	36.4± 2.5	37.4± 2.6	36.2 ±3.1	36.8 ±2.7
Birth weight [grams]	3150±416.4	2699±775	2889± 769	2411± 900	2450± 94
Apgar score [1-minute]	6.9±0.8	4.1± 1.1	2.6± 1.3	**1.3± 0.7***	**2.4 ±1.5***
Apgar score [5-minute]	8±0.7	5.6± 0.5	4.3± 1.3	**2.7 ±1.1***	**3.6± 1.8***
Umbilical artery pH	7.25±0.84	7.1 ±0.06	**7.04 ±0.12***	**6.86± 0.9****	**6.9 0. ±14****
Umbilical arterybasedeficit [m mol/L]	5.3±2.03	-11.0± 1.7	**-15.5± 4.5***	**-19.7 ±3.9***	**16.5± 5.1***
NSE [µg/L]	15.8±2.03	15.4 ±8.2	**33.9 ±16.2****	**46.8± 19.5****	**61.5 ±18.7*****

M mol/L: millimolecule /L **NSE:** neuron specific enolase **µg/L** microgram/ Liter*: P<0.5**,** **: p<0.01***: P<0.001

Table [IV]: Outcome and neurologic sequels in 3 groups of HIE after follow –up [neonatal period, 6 month and 12 month of age], presented as number [n] and percentage [%].

Outcome	Mild HIE n=9		Moderate HIE n=13		Severe HIE n=8		Total n=30	
	N o	%	N o	%	N o	%	N o	%
1.Dead,n=3	**0**	**0**	**1**	**8**	**2**	**25***	**3**	**10**
2.Alive, n= 27	**9**	**100**	**12**	**82***	**6**	**75***	**27**	**90**
neonatal clinical seizures	**0**	**0**	**4**	**33.3***	**5**	**62.5***	**9**	**33.3**
post- neonatal epilepsy	**0**	**0**	**2**	**15.4***	**4**	**50***	**6**	**22.2**
normal neurologic evaluation on 6 mo&12 mo.	**9**	**100**	**3**	**25***	**1**	**16.6***	**4**	**22.2**
3.Adverse outcome [severe motor deficit],n=7	**0**	**0**	**2**	**16.6**	**5**	**83.3***	**7**	**26**

HIE: hypoxic ischemic encephalopathy **mo:** month, ***:** significant increased compared with those with mild HIE**.**

Table [I]shows that there was no statistically significant difference [P>0.05] of gestational age, birth weight and gender. There was a significant difference [P<0.05] between delivery routes in the two groups. The majority of healthy neonates [60%] were delivered via the vaginal route. Meanwhile, the majority of asphyxiated infants [57%] were delivered by C/S. The mean Apgar scores were significantly higher in control neonates than that in asphyxiated neonates, through all periods. The mean arterial cord blood pH was significantly lower in asphyxiated neonates than that in control neonates. Meanwhile, the mean arterial cord blood base deficit was significantly higher in asphyxiated neonates than that in control neonates. The mean serum NSE was significantly higher in asphyxiated neonates compared with that in control neonates. All control neonates were alive, while3 out of 30 asphyxiated neonates [10%] died.

Upon connection between serum NSE and each of gestational age, birth weight, Apgar scores, umbilical artery pH and umbilical artery base deficit in suffocated neonates we discovered non-significant correlation with each of gestational age, birth weight and umbilical artery pH. . Meanwhile, there was a significant negative correlation [P<0.05] between serum NSE and Apgar scores and a significant positive correlation between serum NSE and umbilical artery base deficit [Table II].

Clinical and biochemical perinatal characteristics of 30 asphyxiated neonates versus 20 healthy control neonates demonstrated that; gestational age and birth weight were non-significantly different in both asphyxiated neonates and control neonates. Apgar scores [at 1-minute and 5-minutes] were significantly lower, in neonates with HIE grades II&III and those asphyxiated neonates with neurological sequalae, than that in control neonates. Umbilical artery pH was significantly lower in HIE grade II neonates and very highly significant in HIE grade III neonates and those with neurological sequalae, than that in control group. The mean umbilical artery base deficit was significantly higher in HIE grade II&III neonates and in these with neurological sequalae. The mean serum NSE was highly significantly increased in HIE grade II neonates and very highly significantly increased in HIE grade III neonates and in those with neurological sequalae than that in control neonates [Table III].

Neurologic outcome of 30 asphyxiated neonates:

Table IV shows that the cohort, 3 of 30 infants [10%] with neonatal HIE[moderate and severe] died in the NICU; one within 3 days of life with multi-organ failure and the other two who had suppression of brain stem at 10 days of life after respiratory arrest.

Out of 27 survivors 9 neonates [33.3%] had neonatal clinical seizures in the NICU. Six of them received only phenobarbital, whereas the rest received phenobarbital, phenytoin, and midazolam for refractory neonatal seizures. On discharge from the NICU, all neonates with mild HIE had normal neurologic examination. Whereas, out of 18 neonates [as 3 died in NICU], 4 neonates [22.2%] with moderate to severe HIE had normal neurologic examination. These 4 neonates also had normal neurologic and developmental assessment on 6 months, and 12months.

Post-neonatal seizure were seen in 6 of 27 [22%] neonates [2 of them had moderate HIE&4 babies had severe HIE].

At 12 months age, adverse neurological outcome was reported in 7 of 27 [26%] survivors, in the form of severe motor deficit [spastic tetraparesis in 4 newborn, spastic diparesis in 2 and spastic hemiparesis in one infant].All of these infants had moderate to severe HIE.

A fatal outcome was significantly observed among neonates suffering severe HIE, neonatal clinical seizures and post-neonatal epilepsy were significantly observed among neonates who suffer moderate-to-severe HIE. Adverse neurological outcome with severe motor deficit [cerebral palsy] was significantly more observed among neonates suffering severe HIE.

DISCUSSION

Hypoxic- ischemic encephalopathy describes the abnormal neurological state occurring in the neonates following a significant hypoxic- ischemic insult. The insult may occur antenatally, intrapartum or less commonly postnatally [9].Clinical grading of HIE, which has been proposed as a highly sensitive method of predicting death or severe handicap after perinatal asphyxia, has its limitations [10].

In an attempt to determine the cerebral injury and to predict the neurologic outcome, various investigations have been utilized as an adjunct to the clinical examination. During the last decade biochemical indicators of brain damage have been investigated after asphyxia [4]. Enolase, a glycolytic enzyme, has been accounted for as a biochemical index of neuronal damage [10]. HIE after perinatal asphyxia is associated with increased CSF and/or serum concentrations of brain- specific biochemical markers, of those brain specific proteins, the NSE [5]. Thus the aim of this study was to evaluate serum concentration of NSE, as a marker of the severity of HIE and to elucidate the relation among the concentration of NSE, grade of HIE and long- term outcome.

In this study, the mean serum NSE of HIE group [41.8 µg/L] was significantly higher than that in control group [15.8 µg/l], [P=0.003].Similar results were gotten by different studies [5,11,12,13]. **Tekgul et al** [12] added that the concentration of NSE, in CSF, was significantly higher in neonates with moderate to severe HIE than that in control healthy neonates and concluded that the presence of raised NSE values in serum and CSF after perinatal asphyxia can be a sensitive indicator of prominent brain damage.

In this study, upon correlation between the mean serum levels of NSE and each of gestational age, birth weight, Apgar score [at 1

&5 minutes], umbilical artery base deficit and umbilical artery blood pH among asphyxiated infants, we found non- significant correlation between serum NSE and each of gestational age birth weight and umbilical artery blood pH .On other hand, there was a significant negative correlation between the mean serum NSE and Apgar scores. In addition, there was a significant positive correlation between the mean serum NSE and umbilical artery blood base deficit. Similar result was obtained by **Nagdyman et al** [14].

The degree of brain injury is the major determinant of the final outcome. The severity of HIE is usually classified as mild [Stage I HIE], moderate [Stage II HIE], or severe [Stage III HIE].According to Sarabat & Saranat [6], 9 [30%] infants in our HIE group were classified as in Stage I, 13 [43%] infants as in stage II and 8 [27%] infants as in stage III.

In the present study, the overall outcome was consider adverse in 10 of 30 [33%] neonates with HIE. Seven of 27 [26%] survivors developed severe motor deficit [spastic tetraparesis in 4 newborn, spastic diparesis in 2, and spastic hemiparesis in 1] all of these neonates had moderate to severe HIE. Post neonatal seizures were seen in 6 of 27[22%] infants. In follow-up three of them had post neonatal epilepsy and were set in the group of neonates with "adverse" outcome at 12 months of age. All of them also had severe motor deficit on neurologic examination.

Ten of 13 [77%] neonates with moderate to severe HIE had an adverse outcome, For the remaining three neonates, two [15%] had normal neurologic outcome at 12 months old and the other [8%] had gross motor delay with normal neurologic examination. A favorable outcome was seen in all neonates with mild HIE. Seven of the 9 neonates with mild HIE [78%] had normal neurologic examination at 12 months old. The remaining two neonates had gross motor delay with normal neurologic examination.

In this study NSE was observed to be significantly higher in asphyxiated neonates with adverse neurological outcome or death, in contrast with those with favorable outcome. Several other studies also showed that serum NSE could be predictive of the degree of HIE and poor outcome [15].On the other hand, different studies [16,17,18] reported a strong relationship between concentrations of NSE in CSF and severity of stage of HIE, extent of brain damage and subsequent neurological outcome, but there was no clear association between it and serum NSE.

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