International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: 6.614
Available Online at www.journalijcar.org
Volume 9; Issue 05(B); May 2020; Page No.22103-22111
DOI: http://dx.doi.org/10.24327/ijcar.2020.22111.4356

Research Article

EVALUATION OF SERUM NEURON SPECIFIC ENOLASE AND OTHER BIOCHEMICAL MARKERS IN NEONATAL HYPOXIC ISCHEMIC ENCEPHALOPATHY

[1]*Banashree Swain, [1]Pradeep Kumar Jena and [1]Mangal Charan Murmu

[1]*Assistant professor, Department of Biochemistry, S C B Medical College, Cuttack
[1]Associate professor, Department of Pediatrics, S C B medical college, Cuttack

A R T I C L E I N F O

Article History:
Received 10th February, 2020
Received in revised form 2nd March, 2020
Accepted 26th April, 2020
Published online 28th May, 2020

Key words:
Apgar Score, Hypoxic-Ischemic Encephalopathy, Enolase, Creatine Kinase

A B S T R A C T

Introduction : Perinatal asphyxia is a challenge to the survival of the fetus or the newborn due to lack of oxygen (hypoxia) and/or lack of perfusion (ischemia) to various organs. In a country like India when home deliveries are predominant the infant mortality rate is also equally high. The common denominator of hypoxic ischemic injury is deprivation of the supply of oxygen to the central nervous system. An oxygen deficit may be incurred by either hypoxemia or ischemia. Hypoxemia is defined as a diminished oxygen content of the blood and ischemia is characterized by reduced perfusion of that particular tissue; generally the two tend to occur simultaneously or in sequence. Asphyxia is an impairment of gas exchange that results not only in the deficit of oxygen in blood but also an excess of carbon dioxide causing acidosis. The acidosis further leads to hypotension and ischemia culminating in hypoxic-ischemic injury. Hypoxic- ischemic encephalopathy (HIE) invariably leads to permanent damage to CNS tissues that may result in neonatal death or manifest later as cerebral palsy or developmental delay. **Aim &objective:** To estimate and compare the serum levels of Neuron specific enolase (NSE), Creatine Kinase-Muscle Brain fraction (CK-MB), Lactate Dehydrogenase (LDH), SGOT, SGPT, urea, creatinine in HIE and non-HIE term neonates. **Method:** This prospective study was conducted from December 2017 to June 2019 study in the department of Biochemistry and department of Pediatrics Department of Pediatrics, S C B Medical College, Cuttack. **Observation:** Mean serum level of neuron specific enolase (NSE) among control group of neonate was 16.65ng/ml with a 95% CI of 15.02 ng/ml to 18.27 ng/ ml with a width of 1.63 ng/ml which is statistically significant from the mean serum level of the case group of neonate. The diagnostic performance of CK-MB in differentiating HIE from non-HIE is excellent with a sensitivity of 78.6%, specificity and PPV of 100%, a NPV of 82.4% and area under ROC curve of 0.968 when the sample was drawn at 6-12 hours of life. **Conclusion:** The biochemical marker like CK-MB,LDH and NSE can help to institute early neuroprotective measures for better neonatal outcome in case when proper history of hypoxic ischemic encephalopathy is not available.

INTRODUCTION

When a newborn baby passes through a state of hypoxia and ischemia the baby is vulnerable to develop condition like encephalopathy. It is amply evident from the clinical assessment of the baby that when neonate fails to initiate and sustain breathing after delivery, the baby is likely to suffer from hypoxic ischemic encephalopathy (HIE) [1, 2]. According to latest estimates of WHO approximately 4 million babies die each year before the age of one month [3]. Ninety eight percent of these neonatal deaths take place in developing countries. According to NNPD 2000 data perinatal asphyxia was responsible for 20% of all neonatal deaths [4].

Corresponding author:* **Mangal Charan Murmu,
Associate Professor , Department of Pediatric , S C B Medical college , Cuttack

About 25-50% of infants with HIE die in the neonatal period [5] and 25-60% of the survivors are left with permanent neurodevelopmental abnormalities[6]. Perinatal asphyxia refers to an impairment of the normal oxygenation during parturition and the ensuing adverse effects on the fetus/neonate. In India, between 250,000 to 350,000 infants die each year due to perinatal asphyxia, mostly within the first three days of life. In addition, ante-partum and intra partum asphyxia contributes to as many as 300,000 to 400,000 stillbirths [7].

The outcomes of HIE are devastating and permanent making it a major burden for the patient, family and society. It is critical to identify and develop therapeutic strategies to reduce brain injury in newborns with asphyxia. Early assessment of the severity of an acute cerebral lesion secondary to hypoxic ischemic encephalopathy may provide a very useful basis for

preventive or therapeutic decisions in pediatric patients. Some authors have suggested that biochemical indicators may be more effective than the results of clinical evaluation, Apgar score [8], pH of cord blood, EEG and neuroimaging data[9].In earlier studies, several biochemical markers like lactate/creatinine ratio, neuron-specific enolase (NSE), acidic protein, uric acid, brain-specific creatine kinase were investigated in the blood or cerebrospinal fluid of infants with perinatal asphyxia[10,11]. Urinary uric acid/creatinine ratio and NSE are well established bio markers in predicting the severity and outcome of perinatal asphyxia [11,12].

HIE is a condition in which serum concentration of brain specific biochemical markers may be elevated. Neuroprotective intervention in asphyxiated newborn requires early indicators of brain damage to initiate therapy [12]. Estimation of neurophysiological markers such as brain specific creatine kinase (CK-BB), brain specific lactic dehydrogenase isomer, glutamate and neurone specific enolase in the CSF are all useful in predicting both the immediate dysfunction and the long term outcome[13,14,15].

A "therapeutic window" exists in the early hours following asphyxia and perhaps for a longer period, when intervention can attenuate activation of the neurotoxic cascade that leads to ultimate cell death, either hours or days later [16].

Neuron-Specific Enolase (NSE) is significantly increased in the infants with HIE and correlated well with poor out come in neonate having very high level of NSE (median value 25.4 micrograms/l) compared with control infants (10.0 micrograms/l) [9]. Verdú Pérez A *et al* found that, the presence of elevated NSE values in blood after perinatal asphyxia can be a sensitive indicator of conspicuous brain damage[17].

Several workers have previously demonstrated that neuron specific isomers of creatine kinase (CK) and lactate dehydrogenase (LDH) are released into circulation following anoxic injury from many tissues of their storage and are reflected in total serum levels [13,14]. The sites of storage of CK are brain (CKBB), skeletal muscle (CKMM), the heart muscle (CKMB) and the sites of storage of LDH are the brain, the kidney, the heart, skeletal muscle and the erythrocytes.

Both CK and LDH are raised in birth asphyxia and are more marked among those who developed HIE [18]. Serum CK-MB, LDH, SGOT, SGPT have been studied to differentiate asphyxiated from non-asphyxiated neonates who presented with non specific signs of sickness [19]. Leakage of intracellular enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) signaling multi-organ dysfunction is seen together with HIE after perinatal asphyxia [20,21,22]

Therefore owing to increasing relevance as mentioned above the present study is designed to compare the serum levels of NSE, CK-MB and LDH among asphyxiated and non asphyxiated term neonates and to ascertain whether these enzymes can distinguish an asphyxiated from a non asphyxiated neonate and can predict their severity and outcome or not.

AIMS AND OBJECTIVES

To estimate and compare the serum levels of Neuron specific enolase (NSE), Creatine Kinase-Muscle Brain fraction (CK-MB), Lactate Dehydrogenase (LDH), SGOT, SGPT, urea, creatinine in HIE and non-HIE term neonates . 2. To ascertain whether the above biochemical markers can distinguish HIE from non-HIE in case of non-specific neonatal sickness with no proper perinatal history. 3. Any correlation of the above mentioned parameters with the severity of HIE and their final outcome.

MATERIALS AND METHOD

This prospective study was conducted from December 2017 to June 2019 study in the department of Biochemistry and department of Pediatrics Department of Pediatrics, S C B Medical College, Cuttack after approval of institutional ethical committee Two groups of neonates were included in the study. One group consisted of cases of HIE selected from the department of Pediatrics of S C B Medical College, Cuttack diagnosed on the basis of antenatal, natal, postnatal history physical examination, apgar score at one minute.

The other group was the control group that included age and sex matched otherwise healthy neonate admitted for hyperbilirubinemia received only phototherapy. All the data regarding the history, clinical examination and laboratory investigation will be recorded in a proforma.

Inclusion Criteria

Case

Cases of HIE diagnosed according to American academy of Pediatrics (AAP). All the following must be present for designation of asphyxia. 1.Profound metabolic or mixed acidemia pH < 7.00 in cord blood. 2.Persistence of Apgar score 0-3 for longer than 5min. 3.Neonatal neurological sequelae (seizure, coma, hypotonia). 4. Multiple organ involvement eg: kidney, lungs, heart, intestine.

Control

The control group consists of age and sex matched otherwise healthy newborn receiving only phototherapy for neonatal hyperbilirubinemia.

Exclusion Criteria

Case: HIE patients with: 1.septicemia, 2.meconium aspiration syndrome, 3.associated congenital anomaly, 4.inborn error of metabolism.

Control: 1.Presence of any sickness or complication other than hyperbilirubinemia 2.Hyperbilirubinemia with abnormal LFT After institute ethical committee approval, term neonates with umbilical cord blood pH < 7 and Base deficit > 16meq, APGAR < 5 at 10 min and any one of the following viz: evidence of encephalopathy, evidence of fetal distress, assisted ventilation for at least 10 min after birth, evidence of any organ dysfunction, history of acute perinatal event were included as cases. Babies with major congenital abnormalities and extramural babies were excluded from the study. All babies were managed as per standard guidelines. Babies were enrolled after written informed consent from the parent.

Two ml of venous blood was collected in sterile bottle under aseptic condition within 72hrs after birth and serum was separated after centrifugation. Serum was stored at -20^0C for estimation of NSE, CPK-MB, LDH, SGOT, SGPT,Urea, Creatinine.

Investigations Done

Along with routine investigation like CBC(complete blood count), CRP,RBS, CXR PA view, Micro ESR, Band cell count following investigation were done.
Serum Neuron specific enolase (NSE) by Electrochemiluminescence immunoassay "ECLIA" .2. Serum Lactate dehydrogenase (LDH) by enzymatic method (pyruvateto lactate) Spectrophotometry .3. Serum SGOT, SGPT (kinetic method Spectrophotometry) .4. Serum Creatine kinase (CPK-MB) by UV kinetic method activated NAC .5. Serum urea Urease-end point method,6. Serum creatinine by Modified Jaffes

Statistical Methods

Descriptive statistical analysis has been carried out in the present study. Results on continuous measurements are presented as Mean ± SD, 95% CI (lower and higher bound) and results on categorical measurements are presented in Number (%). Significance is assessed at 5% level of significance. Student t test (two tailed, independent) has been used to find the significance of study parameters on continuous scale between two groups (Inter group analysis) and the Chi-square test has been used to find the significance of study parameters on categorical scale between two or more groups. ROC Curve analysis has been performed to find the diagnostic performance of NSE, CK-MB and LDH.

Diagnostic statistics

		Disease			
Test	**Present**	**N**	**Absent**	**N**	**Total**
Positive	True Positve	a	False Positive	c	a+c
Negative	False Negative	B	True Negative	d	b+d
Total		a+b		c+d	

The following statistics can be defined

Sensitivity: probability that a test result will be positive when the disease is present (true positive rate, expressed as a percentage).= a/(a+b)
Specificity: probability that a test result will be negative when the disease is not present (true negative, expressed as a percentage). = d / (c+ d)
PPV(Positive predictive value): This is calculated as True Positive/True Positive + False Positive i.e.(a/a +c)
NPV(Negative predictive value): True Negative/False Negative +True Negative i.e. (d / b + d)

Statistical software

Statistical analysis was done by statistical software SPSS for windows version 21. P values were calculated using chi-square test and ANOVA (analysis of variance) test. P< 0.05 was considered as significant and P<0.001 as highly significant. The Microsoft word, Excel, Sigma plot and XLSTAT 2012 has been used to generate graphs, tables etc.

Observations

In the present study a total of 100 neonates were enrolled, out of which 50 were cases [HIE, (HIE-I=12, HIE-II=23, HIE-III=15)] and another 50 neonates were controls which were otherwise healthy but admitted for neonatal hyperbilirubinemia to receive phototherapy. Cases and controls were strictly taken as per inclusion and exclusion criteria mentioned in Materials and Methods. Various clinical and biochemical parameters and their results were analyzed, statistically scrutinized and compared.

Table 1 Distribution of Gender in Cases and Controls

Gender	**Controls (n=50)**	**Case (n=50)**		
		HIE-I	**HIE-II**	**HIE-III**
Male(%)	31(62%)	7(14%)	13(26%)	10(20%)
Female (%)	19(38%)	5(10%)	10(20%)	5(10%)
Total (%)	50(100%)	12(24%)	23(26%)	15(30%)
Inference	Gender distribution among control group and cases are not statistically significant (p=1)			

Male neonates contributed 31(62%) among controls and 30(60%) among cases where as females contributed 19(38%) among controls and 20(40%) among cases, with a male : female ratio of almost 1.6:1 in both cases as well as controls Gender distribution of neonates is statistically similar between two groups as evidenced by p value of 1.

Table 2 Different Modes of Delivery in Cases and Controls

Mode of delivery	**Control No(%)**	**Cases no (%)**			**Total No(%)**
		HIE-I	**HIE-II**	**HIE-III**	
NVD	36 (72%)	8(16%)	11(22%)	7(14%)	62(62%)
LSCS	12(24%)	3(6%)	9(18%)	5(10%)	29(29%)
ID	2(4%)	1(2%)	3(6%)	3(6%)	9(9%)
TOTAL	50	12	23	15	100

Normal vaginal delivery was the mode of delivery in 62% followed by LSCS in 29% and instrumental delivery in 9% of neonates. Incidence of HIE among neonates delivered by instrumental delivery is significantly higher (p=0.02) than the NVD and LSCS group where as HIE risk is higher in LSCS group as compared to delivery by NVD group but is not statistically significant (p=0.06).

Table 3 Birth Weight of Cases and Controls

Birth weight	**Control No(%)**	**Cases No(%)**			
		HIE-I	**HIE-II**	**HIE-III**	**TOTAL**
2.5-<3Kg	14(28%)	8(16%)	4(8%)	2(4%)	14(28%)
3-<3.5kg	29(58%)	6(12%)	11(22%)	11(22%)	28(56%)
>3.5kg	7(14%)	2(4%)	4(8%0	2(4%)	8(16%)

Distribution of birth weight among cases and control groups reveals that 28% of both control and case group was constituted by neonates having 2.5 Kg-3.0 Kg birth weight where as 58% of control group and 56% of case group had neonates with birth weight 3-3.5 Kg and only 14% of control group and 16% of case group constituted neonates of > 3.5 Kg. Distribution of birth weight among cases and control groups are not statistically different from each other (p = 0.893).

Table 4 Parity of the Mother of the Neonate in Cases & Controls

Parity of the mother	**Control No(%)**	**Cases No (%)**			
		HIE-I	**HIE-II**	**HIE-III**	**TOTAL**
Primipara	24(48%)	8(16%)	11(22%)	10(20%)	29(58%)
With 1 sibling	17(34%)	3(6%)	9(18%)	3(6%)	15(30%)
With ≥2 sibling	9(18%)	1(2%)	3(6%)	2(4%)	6(12%)

Among the control group of 50 neonates 24(48%) were born to primi mother followed by 17(34%) to mother with one child and only 9(18%) were born to mother with two or more children. Among the 50 neonates in the case group 29(58%) were born to primi mother followed by 15(30%) to mother with one child and 6(12%) were born to mother with two or more children. Proportion of primi and multi gravida mothers were statistically similar in both the group with p=0.55.

Table 5 Gestational Age of Neonates in Cases & Controls

Gestational Age	Control No(%)	Cases No(%) HIE-I	HIE-II	HIE-III	TOTAL
≤37 weeks	12(24%)	4(8%)	3(6%)	2(4%)	9(18%)
38-40 weeks	29(58%)	8(16%)	19(38%)	12(24%)	39(78%)
>40 weeks	9(18%)	0(0%)	1(2%)	1(2%)	2(4%)

Majority of the neonates i.e, 58% in control group and 78% in the case group were having gestational age 38-40 weeks where as only 24% in control group and 18% in case group had gestational age < 37 weeks. 18% of neonates of control group and 4% of case group had gestational age 40 weeks (p=0.042)

Table 6 Average Sampling Time in Control and Cases

Sampling time in hours	Control Mean ±SD	Cases Mean ±SD HIE-I	HIE-II	HIE-III	TOTAL
Average	38.62	39.83	36	38.67	37.72
SD	21.02	21.98	20.69	26.24	21.6
Earliest	6	6	7	6	6
Latest	72	70	70	72	72

Mean sampling time in the control group was 38.62 hrs where as in case group it was 37.72 hrs. Earliest time at which sample was collected in control group was 6 hours of life which is same as that of case group. In other wards the timing of sampling in case group was not different from control group.

Table 7 Serum Transaminases Level in Cases & Controls With Their Descriptive Statistics

Parametre		Control	HIE-I	HIE-II	HIE-III	TOTAL
Serum AST(U/L) (Case)	Mean	36.62	71.17	82.3	104.8	73.43
	SD	17.53	69.04	77.61	38.92	97.84
	95%CI Upper	31.64	60.24	27.3	83.25	31.12
	95%CI Lower	41.6	104.35	115.03	126.35	115.73
	Width	4.98	22.06	43.87	21.55	42.31
Serum AST(U/L) (Control)	Mean	20.24	34.45	46.37	67.2	39
	SD	9.16	20.43	41.92	28.63	52.48
	95%CI Upper	17.64	35.45	21.47	51.35	16.31
	95%CI Lower	22.84	58.28	47.43	83.05	61.69
	Width	2.6	11.42	12.98	15.85	22.69

Mean serum level of AST in control is 36.62 U/L where as in cases the mean is 82.30 U/L. Mean serum level of AST in case group is statistically different from the mean serum level in control group (p = 0.001).

Mean serum level of ALT in case group is 46.37U/L which is statistically different from mean serum level of ALT in control group of 20.24 U/L with a p value of 0.001

Table 8 serum urea & creatinine level in cases & controls with their descriptive statistics

Parametre		Cases Control	HIE-I	HIE-II	HIE-III	Total
Serum Urea (mg/dl)	Mean	27.7	40.07	71.91	59.04	60.57
	SD	7.26	11.12	23.8	17.48	22.95
	95%CI Upper	25.64	35.69	61.62	49.38	54.05
	95%CI Lower	29.77	47.82	82.2	68.72	67.09
	Width	2.07	6.07	10.29	9.68	6.52
Serum Creatinine (mg/dl)	Mean	1.01	0.53	1.17	1.5	1.12
	SD	0.82	0.29	0.55	0.57	0.61
	95%CI Upper	0.87	0.35	0.93	1.19	0.94
	95%CI Lower	1.25	0.72	1.4	1.82	1.29
	Width	0.24	0.19	0.24	0.32	0.18

Mean serum level of urea in control group is 27.04mg/dl which is statistically different from the mean serum level of urea in case group of 60.57mg/dl with a p value of 0.001.
Mean serum level of creatinine in case group is 1.12mg/dl which is statistically not different from the mean of control group of 1.01mg/dl (p=0.48).

Table 9 Serum Ck- Mb (U/L) Level In Cases & Controls With Their Descriptive Statistics

Subject	Mean	SD	95% CI Lower	95% CI Higher	Width
Contrl	26.39	9.05	23.78	28.93	2.58
Case as a whole	94.47	126.72	58.46	130.48	36.01
HIE-I	104.08	45.02	75.48	132.69	28.61
HIE-II	74.33	71.55	43.39	105.27	30.94
HIE-III	171.33	193.4	64.22	278.43	107.11

Mean serum creatinine kinase-muscle brain(CK-MB) fraction level in control group was 26.36 U/L with a 95% confidence interval level of 23.76 to 28.93 U/L as compared to 94.47 U/L in case (HIE) group with a 95% CI of 58.46 to 130.48 U/L. The mean serum level of CK-MB in case group is statistically different from the control group with a p value of 0.001. Mean CK-MB level of control group, case(HIE) group, HIE-1,HIE-2, HIE-3 groups are statistically different from each other except the level in HIE-2 & HIE-3 which is not different from one another as evidenced by p value of 0.08.

Table 10 Serum Ldh (U/L) Level in Cases & Controls With Their Descriptive Statistics

Subject	Mean	SD	95% CI Lower	95% CI Higher	Width
Control	441.75	170.7649	393.23	490.29	48.53
Case as a whole	1238.4	982.4704	959.19	1517.62	279.215
HIE-I	534.826	149.0752	440.12	629.55	94.715
HIE-II	1360.826	1109.255	881.15	1840.5	479.675
HIE-III	1613.533	909.7558	1109.73	2117.34	503.805

Among the control group the mean serum level of LDH was 441.76 U/L with a 95% CI of 393.23 U/L to 490.29 U/L and a width of 48.53 U/L which is statistically different from the mean serum level of LDH among the case group being 1238.4U/L with a 95% CI level of 959.19 U/L to 1517.62 U/L and a width of 279.22 U/L as evidenced by p value of 0.001. Mean serum level of LDH is statistically different from one another among control, case group, HIE-I, HIE-II and HIE-III, except HIE-II & III where they are not different as the p value is 0.47 (just like CK-MB)

Table 11 Serum Neuron Specific Enolase (Nse) Level (Ng/Ml) In Cases & Controls With Their Descriptive Statistics

Subject	Mean	SD	95% CI Lower	95% CI Higher	Width
Control	16.65	5.72	15.02	18.27	1.63
Case as a whole	52.45	23.71	45.71	59.19	6.74
HIE-I	30.65	9.35	24.71	36.59	5.94
HIE-II	52.79	17.94	45.03	60.55	7.76
HIE-III	69.36	26.08	54.92	83.8	14.44

Mean serum level of neuron specific enolase (NSE) among control group of neonate was 16.65ng/ml with a 95% CI of 15.02 ng/ml to 18.27 ng/ ml with a width of 1.63 ng/ml which is statistically significant from the mean serum level of the case group of neonate ie. 52.45ng/ml with a 95% CI of 45.71 to 59.19ng/ml and a width of 6.75ng/ml as evidenced by a p value of 0.001. The mean serum level of NSE within different HIE group as well as case and control groups are different statistically from each other and unlike serum LDH and CK-MB here the difference between HIE-II & HIE-III groups are also statistically significant as the p value is 0.03.

Table 12 Sensitivity, Specificity And Predictive Values Of Ck-Mb, Ldh And Nse

Marker	Cut off	Sensitivity (95%CI)	Specificity (95%CI)	PPV (95%CI)	NPV(95%CI)	ROCAUC
CKMB(U/L)	92.6	24(12-36)	100	100	57(43-71)	0.704
LDH(U/L)	580	64(51-77)	76(64-88)	73(61-85)	68(55-81)	0.81
NSE(ng/ml) Case	30	80(69-91)	98(94-100)	98(94-100)	83(73-93)	0.982
NSE (ng/ml) Control	40	84(72-86)	98(95-100)	98(95-100)	90(83-97)	

At cut off level of 92.6 U/L, CK-MB has 24% sensitivity with 95%CI is from 12 – 36 U/L, 100% specificity, 100%PPV and 57% NPV with 95%CI 43-71% and an area of 0.704 under ROC curve. So it will be a good diagnostic test but will not be a good screening test. Serum LDH at 580 U/L cut off level has a sensitivity of 64% with 95%CI of 51-77%, 76% specificity with 95%CI of 64-88%, PPV of 73% and NPV of 68% and an area of 0.81 under the ROC curve. Serum NSE at 30 ng/ml of cut off value has a sensitivity of 80%, specificity of 98%, PPV of 98% and 83% NPV with an area of 0.982 under ROC curve making NSE a very good tool for differentiating HIE from non- HIE.

Table 13 Ultimate Outcome of the Cases and Controls

Study group		Outcome		
		Recovery without sequela	Recovery with sequel & need AED	Death
Control(NON HIE}		50(100%)	0	0
Cases HIE	HIE-I	11(91.6%)	1(8.3%)	0
	HIE-II	6(26.1%)	15(65.2%)	2(8.7%)
	HIE-III	1(6.7%)	7(46.7%)	7(46.7%)

In the present study all the patient in control group has recovered. Out of the case group 91.6% of mild grade HIE (HIE-I) has recovered completely without any sequela and only one patient (8.3%) recovered with few neurological deficit and needed antiepileptic drug (AED) at the time of discharge. Among the moderate encephalopathy (HIE-II) group 26.1% has recovered without any sequel where as majority(65%) developed neurological sequelae of one or other form and needed AED at the time of discharge. Only two patient (8.7%) died among HIE-II in spite of all standard care. Out of all the HIE-III group only one patient could be discharged home without any neurological deficit and AED could be successfully weaned prior to discharge. However 46.7% of HIE-III developed neurological sequelae and needed AED at the time of discharge and another 46.7% died in spite of all standard treatment.

Table 14 Outcome of the Cases and Controls Correlated with Their Corresponding Mean Serum Nse Levels

Study group with outcome		NSE (ng/ml)	
		Mean	Percentile 95
NON HIE	Recover without sequel	16.65	28.90
	Recovery with sequelae & need of AED	-	-
	Death	-	-
HIE -I	Recover without sequel	28.47	41.49
	Recovery with sequelae & need of AED	54.70	54.70
	Death	-	-
HIE-II	Recover without sequel	35.52	48..94
	Recovery with sequelae & need of AED	55.02	81.45
	Death	87.86	103.76
HIE-III	Recover without sequel	24.54	24.54
	Recovery with sequelae & need of AED	55.05	68.41
	Death	90.07	123.45

Above table has nicely shown the correlation of severity of the outcome with the mean serum NSE level. The mean serum level of NSE was found to be 85-90 ng/ml among those died out of complication of HIE where as it was 55ng/ml in those developed neurological sequelae needing AED at time of discharge irrespective of the fact whether it belongs to HIE-I, HIE-II or HIE -III. The mean serum NSE was 35ng/ml and below in those who recovered completely without any neurological sequel and without any need of AED. Box plot diagram has beautifully shown the above correlation of high serum NSE level with poor outcome in HIE irrespective of grades of HIE.

DISCUSSION

Major cause of infant mortality rate is neonatal death which in turn is mostly due to hypoxic ischemic encephalopathy (HIE) in addition to prematurity and sepsis. Early institution of neuroprotective measures can reduce the mortality as well as morbidity due to HIE for which early diagnosis and prediction of progression from birth asphyxia to HIE is equally vital. The present study was conducted to ascertain whether different biochemical markers like CK-MB, LDH, NSE, AST, ALT, Serum urea and creatinine can be good in differentiating asphyxia from non asphyxial etiology.

Males outnumbered females (61% vs 39%) in the present study, even in three different degree of asphyxia (mild-58.3%, moderare-56.5%, severe 66.7%). Male: Female ratio was 1.6 : 1(Table:1). This study is well comparable with the results of Yvonne W. Wu *et al*[23] (1991-2000) who documented the association of ethnicity, male gender and low socio-economic status with increased incidence of birth asphyxia.

In the present study mild, moderate and severely asphyxiated babies constituted 24%, 46% and 30% respectively (Table- 1). M. Ellis & N. Manandhar *et al* [24] (1997) found the incidence to be 32%, 39.5% and 27.5% respectively. Variation in incidence might be due to most of the cases admitted to our hospital were referred cases sent from other peripheral hospitals of Orissa where most of the mild cases were managed. Another reason for the variation might be due to small sample size (n= 100) in comparison to the vast number of cases (n= 14,371) in their study.

Normal vaginal delivery was the mode of delivery in 62% followed by LSCS in 29% and instrumental delivery in 9% of neonates. Incidence of HIE among neonates delivered by instrumental delivery is significantly higher (p=0.02) than the NVD and LSCS group where as HIE risk is higher in LSCS group as compared to delivery by NVD group but is not statistically significant (p=0.06)(Table- 2). Bashir and Zakaria *et al*[25] study showed that incidence of birth asphyxia was more in normal vaginal delivery (40%) & instrumental delivery (30%) as compared to breech(9%), emergency LSCS (21%), elective LSCS . Nadia Badawi *et al*[26] (1993-1995) also observed similar results in their study of 164 term infants. Also similar were the results of the studies conducted by Piyush Gupta *et al* [27] (1997) and A.S Daga *et al* (1990) [28]. Comparative study of baseline characteristics of cases and controls:

Characteristics		Reddy S *et al*		Present study	
		Control(n=20)	Cases (n=25)	Control(n=50)	Cases (n=50)
Gender	Male	90%	64%	62%	60%
	Female	10%	36%	38%	40%
Parity of mother	Primi	35%	52%	48%	58%
	Multi	65%	48%	52%	42%
Mode of delivery	NVD	85%	42%	72%	52%
	Instrumental			4%	14%
	LSCS	15%	58%	24%	34%

Reddy S et a [29]lhad included 90% male & 10% female in control group (n=20) whereas 64% male & 36% female in case group (n=25) in their study whereas in our study we have taken 62% male & 38% female in control group (n=50) and 60% male & 40% female in case group (n=50) . Distribution of male & female in present study is more proportionate than Reddy S *et al* study .

Proportion of primi and multi gravida mothers were statistically similar in both the group with p=0.55 indicating that there was no significant association between birth asphyxia and parity of the mother which is comparable to Reddy S *et al*[24] and Khreisat WH *et al*[30]

Mode of delivery in the present study is well comparable to that of Reddy S *et al* [24] with an exception of 4-14% instrumental delivery included in the present study which was not included in their study.

The present study showed that maximum number of birth asphyxia (56%) were seen in the birth weight between 3.0-3.5 Kg followed by 28%seen in the birth weight of 2.5 – 3.0 Kg and only 16% in the birth weight of > 3.5 Kg (Table- 3). M. Ellis & N. Manandhar *et al* (1997) study showed that 79%cases of severe asphyxia were seen among the babies weighing < 3000 gms; of which 30% were within 2500-2900 gms [24]. The reason for the variation might be due to small sample size (n= 50) in comparison to the vast number of cases (n=14,371) in their study.

Comparative study of Severity of HIE & outcome in cases (HIE)

Severity of HIE & Outcome		Reddy S *et al* (n=25)	Rajkumar PS *et al*(n=30)	Karunatilaka *et al* (n=35)	Present study (n=50)
HIE	Total	-	100%	25.71%	50%
	HIE-I	-	26.67%	14.28%	24%
	HIE-II	-	60%	8.58%	46%
	HIE-III	0%	13.33%	2.85%	30%
	Death	-	16.7%	-	18%

In the present study HIE-I constituted 24%, HIE-II constituted 46% and HIE-III constituted 30% of all cases(HIE) which is statistically comparable to Rajkumar PS *et al*[31] where they had taken 26.67%, 60% and 13.33% respectively in HIE-I,-II &-III; only the HIE-III group was little more in our study which might be due to the fact that our being an tertiary centre we receive all the critically advanced cases more as compared to mild & moderate grade. Karunatilaka DH *et al* [32] in their study had also documented similar incidence where mild grade HIE was more common than moderate and severe grade. We encountered death in 18% of cases majority of which belongs to HIE-3 group which is comparable to 16.7% death noted by Rajkumar PS *et al* [31] in their study.

Mean serum level of ALT in case group is 46.37U/L which is statistically different from mean serum level of ALT in control group of 20.24U/L with a p value of 0.001. Ming-Hung Lin *et al*[33] had also found that mean ALT was 94.22±159.27 in unfavourable group where as it was 59.32±84.24 in the favourable group to develop HIE which was again not statistically significant. Serum AST and ALT is very non specific and value in present study is comparable to that by Ming-Hung Lin *et al* [33].

Mean serum level of urea in control and case group was 27.04mg/dl 60.57mg/dl respectfully where as mean serum level of creatinine in case group and control group was 1.12mg/dl and 1.01 mg/dl which is similar to the levels noted by Ming-Hung Lin *et al*[33] in their study.

Comparative studies of diagnostic performance of Serum CK-MB in HIE

Study group	Sensitivity	Specificity	PPV	NPV	AUROC
Reddy S *et al* CK-MB at 6hours	36%	100%	100%	52%	0.82
Rajakumar PS *et al* CK-MB at 6 hours	56.5%	75.7%	-	-	-
Present study CK-MB	24% (12-36)	100%	100%	57% (43-71)	0.704
Present study CK-MB at 6-12 hours	78.6%	100%	100%	82.4%	0.968

Diagnostic performance of CK-MB , as depicted in the table, is excellent in differentiating HIE from non HIE with a sensitivity of 78.6%, specificity and PPV of 100%, a NPV of 82.4% and area under ROC curve of 0.968 when the sample was drawn at 6-12 hours of life. The sensitivity is reduced to 20% only with the area under curve to 0.704 when the sampling timing is changed to 6-72 hours of life. The parameters are well comparable to the findings of Reddy S *et al*[29] as well as Rajkumar PS *et al* [31].

Comparative studies of diagnostic performance of Serum LDH in HIE

Study group	Sensitivity	Specificity	PPV	NPV	AUROC
Reddy S *et al*	100%	89%	92%	100%	0.998
Karlsson M *et al*	100%	97%	87%	100%	-
Present study LDH at 6-72 hours	64%	76%	73%	68%	0.81
Present study LDH at 60-72 hours	100%	63.6%	68.7%	100%	0.894

The sensitivity, specificity, PPV, NPV and AUROC of present study is almost comparable to that of Reddy S *et al*[29] and Karlsson M *et al*[34] except little decrease in specificity (64% vs 89%) and PPV (69% vs 92%) which is due to the fact that sample was collected from 60-72 hours in our study as compared to 72±2hours by Reddy S *et al*[29] in their study. This was done to increase the duration of time at which we can differentiate HIE from non-HIE by serum LDH and proved to be having reasonably acceptable specificity and PPV with a retained sensitivity and NPV of 100%.

Comparative studies of diagnostic performance of Serum NSE in HIE

Study group	Cut off (ng/ml)	Sensitivity %	Specificity %	PPV %	NPV %	AUROC
Celtik *et al*	40	79	70	-	-	-
Celtik *et al* Poor outcome	45.5	84	84	70-	-	-
Nagdyman N *et al*	46	83	65	42	93	0.763
AD Edwards *et al*	25.5	100	81	52	100	0.942
Present study	30	80	98	98	83	0.982
Present study	40	84	98	95	90	0.982

Sensitivity, specificity, PPV, NPV and area under ROC curve of serum NSE is 84%, 98%, 95%, 90% and 0.982 respectively in the present study indicating an excellent diagnostic performance in differentiating HIE from non-HIE which is well comparable to the findings of Celtik *et al*[18],Nicole N *et al*[35] and AD Edwards *et al*[36]. Celtik *et al* has demonstrated that higher level of serum NSE level(>45.5 ng/ml) is associated with poor outcome in HIE which also has been demonstrated in the present study as association of mean serum level of NSE >55ng/ml with neurological sequelae & need of AED whereas level of >85-90ng/ml with death irrespective of the severity of HIE. In other words serum NSE level when high > 55ng/ml is a better predictor of poor outcome and death in HIE as compared to the severity/grades of HIE. Another finding in present study is that serum NSE level 40ng/ml can predict the HIE to be moderate to severe (HIE II & III).

Summary: This prospective study was conducted from December 2017 to June 2019 study in the department of Biochemistry and department of Pediatrics Department of Pediatrics, S C B Medical College, Cuttack.

A total of 100 neonates were enrolled, out of which 50 were cases (HIE) and another 50 neonates were controls which were otherwise healthy but admitted for neonatal hyperbilirubinemia to receive phototherapy. Blood was collected in sterile bottle under aseptic condition within 72hrs after birth and serum NSE, CPK-MB, LDH, SGOT, SGPT, Urea and Creatinine was estimated in both cases and controls. The Male neonates contributed 31(62%) among controls and 30(60%) among cases where as females contributed 19(38%) among controls and 20(40%) among cases, with a male: female ratio of almost 1.6:1 in both cases as well as controls. Among the case group the HIE-I, HIE-II and HIE-III constituted 12(24%), 23(46%) and 15(30%) of neonates respectively. The normal vaginal delivery was the mode of delivery in 62% followed by LSCS in 29% and instrumental delivery in 9% of neonates. Incidence of HIE among neonates delivered by instrumental delivery is significantly higher (p=0.02) than the NVD and LSCS group. Among control group the birth weight was 2.5-3.0Kg in 28%, 3.0-3.5Kg in 58% and > 3.5 Kg in 14% whereas it was 28%, 56% and 16% in case group respectively. 24(48%) neonates were born to primi mother and 26(52%) to multigravida among control group whereas 29(58%) were born to primi and 21(42%) to multigravida mother among case group. The mean sampling time in the control group was 38.62 hrs where as in case group it was 37.72 hrs with the earliest time to draw the sample was 6 hours and the latest was 72 hours in both case and control group. The mean serum level of AST and ALT was 36.62 U/L and 20.24 U/L in control group whereas it was 82.30 U/L and 46.37U/L among case group which was statistically different (p<0.001). The mean serum level of urea and creatinine was 27.04mg/dl and 1.01mg/dl in control group whereas it was 60.57mg/dl and 1.12 mg/dl among case group respectively. The mean serum creatinine kinase-muscle brain fraction(CK-MB) level in control group was 26.36 U/L with a 95% CI of 23.76 to 28.93 U/L as compared to 94.47 U/L in case (HIE) group with a 95% CI of 58.46 to 130.48 U/L. The mean serum level of CK-MB in case group is statistically different from that of control group with a p value of 0.001. Among the control group the mean serum level of LDH was 441.76U/L with a 95% CI of 393.23 U/L to 490.29 U/L which is statistically different from the mean serum level of LDH among the case group being 1238.4U/L with a 95% CI level of 959.19 U/L to 1517.62 U/L(p < 0.001).The mean serum level of NSE among control group of neonate was 16.65ng/ml with a 95% CI of 15.02 ng/ml to 18.27 ng/ml and a width of 1.63 ng/ml which is statistically significant from the mean serum level of the case group of neonate ie. 52.45ng/ml with a 95% CI of 45.71 to 59.19ng/ml and a width of 6.75ng/ml (p< 0.001).The serum NSE level can be a good biochemical marker in differentiating HIE from control (non HIE) and moderate & severe HIE (HIE-II& HIE-III) from that of mild (HIE-I).The diagnostic performance of CK-MB in differentiating HIE from non-HIE is excellent with a sensitivity of 78.6%, specificity and PPV of 100%, a NPV of 82.4% and area under ROC curve of 0.968 when the sample was drawn at 6-12 hours of life. The sensitivity, specificity, PPV, NPV and AUROC of serum LDH when drawn at 60-72 hours of life in differentiating HIE from non-HIE is 100%, 63.6%, 68.7%, 100% and 0.894 respectively with an excellent differentiating value. Sensitivity, specificity, PPV, NPV and area under ROC curve of serum NSE is 84%, 98%, 95%, 90% and 0.982 respectively indicating an excellent diagnostic performance in differentiating HIE from non-HIE.The serum NSE level of > 55ng/ml is a better predictor of poor outcome and death in HIE as compared to the severity / grades of HIE. The serum NSE level 40ng/ml can predict the HIE to be moderate to severe (HIE II & III).

CONCLUSION

Hypoxic ischemic encephalopathy (HIE) is one of the most common cause of neonatal morbidity and mortality in developing country like India. The signs of HIE are nonspecific and overlap with other common neonatal illness. In the absence of proper perinatal history it is difficult to dignose HIE retrospectively. Early institution of neuroprotective measures can decrease the morbidity as well as mortality due to HIE. Serum levels of CK-MB if drawn at 6-12 hours of birth can differentiate those having HIE from non-HIE. Serum LDH level when estimated at 60-72 hours of birth can statistically differentiate HIE from non-HIE. Serum NSE is an excellent biomarker that is if estimated at any time from 6-72 hours of birth can differentiate HIE from non-HIE with a better diagnostic performance as compared to serum CK-MB and serum LDH. The mean levels of serum urea, creatine, AST, ALT are increased in HIE as compared to control though they have no discriminating capacity between HIE and non-HIE. The serum NSE level 40ng/ml can predict the HIE to be moderate to severe (HIE II & III). Serum NSE level of > 55ng/ml is a better predictor of poor outcome and death in HIE as compared to the predictive capacity of severity /grades of HIE. The results of present study could help the clinician to retrospectively diagnose HIE by estimating the biochemical marker like CK-MB,LDH and NSE when proper

perinatal history is not available and can help them to institute early neuroprotective measures for better neonatal outcome.

Abbreviations

AAP : American academy of paediartics
AED : Anti epileptic drug
AST : Aspartate transaminase
ALT : Alanine transaminase
AUROC : Area under receiver operating curve
CBC : Complete blood count
CK-MB : Creatine kinase muscle brain fraction
CK-BB : Creatine kinase brain fraction
CSF : Cerebrospinal fluid
CRP: C reactive protein
CXR : Chest X ray
EEG : Electroencephalogram
ESR : Erythrocyte sedimentation rate
HIE : Hypoxic ischemic encephalopathy
HR : Heart rate
ID: Instrumental delivery
LDH : Lactate dehydrogenase
LFT: Liver function test
LSCS : Lower segment caesarian section
NNPD : National neonatal and perinatal data base
NSE : Neuron specific enolase
NPV : Negative Predictive Value
NVD: Normal vaginal delivery
PPV : Positive Predictive Value
ROC : Receiver operating curve
SD : Standard deviation
SGOT : Serum glutamate oxaloacetate transaminase
SGPT: Serum glutamic pyruvic transaminase

Bibliography

1. Sarnat HB, Sarnat MS. Neonatal encephalopathy following fetal distress: a clinical and electroencephalographic study. Arch Neurol 1976;33(10):696-705.
2. Namasivayam A, Waldemar A. Carlo, Hypoxic Ischemic Encephalopathy. Nelson Text Book of Pediatrics 19th Edition;569.
3. Costello A, Francis V, Byrne A, Puddephatt C. State of the world's newborns.Kinetik communications 2001.
4. Report of the National Neonatal Perinatal Database(National Neonatology Forum, India)2000.
5. Derganc M, Osredkar D. Hypoxic-ischemic brain injury in the neonatal period.Zdrav Vestn 2008;77:51–8.
6. Miller SP, Ramaswamy V, Michelson D, *et al*. Patterns of brain injury in term neonatal encephalopathy.J Pediatr2005;146:453–60.
7. NNPD. ww.newbornwhocc.org/pdf/nnpd_report 2002-03.PDF. 2003. Accessed 10th April 2013.
8. Apgar V. A proposal for a new method of evaluation of the newborn infant. Curr Res Anaesthes Analges 1953;32(4):260-7.
9. Vilanova Juanola JM, Figueras Aloy J, Palomeque Rico A, Roldán Ros A, Jiménez González R. Marcadores bioquímicos de hypoxia isquemia cerebral. Acta Pediatr Esp 1994;52:422-27 (in Spanish).
10. Vannucci RC, Perlman JM. Intervention for perinatal hypoxic ischemic encephalopathy. Pediatrics 1997;100: 1004-1014.
11. Bader D, Gozal D, Weinger-Abend M, Berger A, Lanir A. Neonatal urinary uric acid /creatinine ratio as an additional marker of perinatal asphyxia. Eur J Pediatr 1995; 154: 747-749.
12. Akisu M, Kultursay N. Value of the urinary uric acid to v creatinine ratio in term infants with perinatal asphyxia. Acta Paediatr Jpn 1998;40: 78-81.
13. Thornberg E, Thiringer K, Hagberg H, Kjellmer I. Neuron specific enolase in asphyxiated newborns: association with encephalopathy and cerebral function monitor trace. Arch Dis Child 1995; 72:39-42.
14. Ekin P, Toet M C, Groeneedaal F, *et al*. Predictive value of neuroimaging, pulse Dopplerand neurophysiology in full term infants with hypoxic ischaemic encephalopathy. Archives of Disease in Childhood 1995; 73: 75-80.
15. Bjerre , Hellstrom- Westas, Rosen , *et al*. Monitoring of cerebral function after severe asphyxia in infancy. Archives of Disease in Childhood 1983; 58: 997-1002.
16. Gluckman P D, Williams C E. When and why do brain cells die? Dev.Med. Child Neurology l992; 34:1010-14.
17. Verdú Pérez A, Falero MP, Arroyos A, Estévez F, Félix V, López Y, Pantoja A, Ureta A (2001) Blood neuronal specific enolase in newborns with perinatal asphyxia. Rev Neurol 32:714–717
18. Çeltik, C., B. Acunaş, N. Öner and Ö. Pala. "Neuron specific enolase as a marker of the severity and outcome of hypoxic ischemic encephalopathy", Bra Dev, 26, 398- 402 (2004).
19. De Praeter C, Vanhaesebrouck P, Govaert P, *et al*. Creatinine Kinase Isoenzyme BB concentrations in the cerebrospinal fluid of newborns.Pediatrics1991; 88:1204-10.
20. Karlsson M(1), Blennow M, Nemeth A, Winbladh B Dynamics of hepatic enzyme activity following birth asphyxia. Acta Paediatr. 2006 Nov;95(11):1405-11
21. Lackmann GM, Tollner U, Mader R. Serum enzyme activities in full term asphyxiated and healthy newborns: enzyme kinetics during the first 144 hours of life. Enzyme & Protein 1993; 47: 160-172.
22. Zanardo V. • Bondio M. • Perini G. • Temporin G.F., Serum Glutamic Oxaloacetic Transaminase and Glutamic-Pyruvic Transaminase Activity in Premature and Full-Term Asphyxiated Newborns. Biol Neonate 1985;47:61–69.
23. Yvonne WW, Backstrand KH. Declining Diagnosis of birth asphyxia in Califernia. Pediatrics 2004;114(6):1584-1590.
24. Ellis M, Manandhar N, Manandhar DS, Del Costello AM. Indian Pediatrics 1998; 35;415-421.
25. Itoo BA, Al-Hawsawi ZM, Khan AH. Hypoxic Ischemic Encephalopathy Incidence and Risk factors in North Western Saudi Arabia. Saudi Med J 2003;24(2):147-153
26. Badawi N, Kurinczuk JJ, Keogh JM. Antepartum risk factors for newborn encephalopathy. BMJ 1998;317:1549-1553.
27. Gupta P, Faridi MMA, Behl D, Agarwal N. Clinical and Biochemical Asphyxia in meconium stained deliveries. Indian Pediatrics 1998;35:353-56.

28. Daga AS, Daga SR, Patole SK. Risk assessment in birth asphyxia. J of Tropical Pediatrics 1990;36(1):34-39
29. Reddy S, Dutta S, Narang A. Evaluation of lactate dehydrogenase, creatine kinase and hepatic enzymes for the Retrospective diagnosis of Perinatal Asphyxia Among Sick Neonates. Indian Pediarics February 17, 2008;45: 144-147
30. Khreisat WH, Habahbeh Z. Risk factors of birth asphyxia; A study at Priince Ali Benn Al- Hussein Hospial,, Jordan. Pakk J Med Sci January- March 20005; 21(1):30-34
31. Rajakumar PS, Vishnu Bhat B, Sridhar MG, Balachander J, Konar BC, Narayanan P, *et al*. Cardiac Enzyme Levels in Myocardial Dysfunction in Newborns with Perinatal Asphyxia. Indian Journal of Pediatrics December. 2008;75: 1223-1225
32. Karunatilaka DH, Amaratunga GWDS, Perera KDNI, Caldera V. Serum creatine kinase and lactic dehydrogenase levels as useful markers of immediate and long- term outcome of perinatal asphyxia.Sri Lanka *Journal of Child Health* 2000; 29: 49-52.
33. MH Lin, HC Chou, CY Chen, *et al* Serum Biomarkers and Outcomes in Asphyxiated Infants Clinical Neonatology 2008 Vol. 15 No.1: 10-15
34. Karlsson M, Wiberg-Itzel E, Chakkarapani E, Blennow M, Winladh B, Thoresen M. Lactate dehydrogenase predicts hypoxic ischemic encephalopathy in newborn infants: a preliminary study. Acta Paediatrica August 2010; 99(8):1139-1144.
35. Nagdyman N, Kömen W, Ko HK, Müller C, Obladen M. Early biochemical indicators of hypoxic-ischemic encephalopathy after birth asphyxia. Pediatr Res. 2001;49:502-506.
36. AD Edwards, P Brocklehurst, AJ Gunn *et al* ;Neurological outcomes at 18 months of age after moderate hypothermia for perinatal hypoxic ischaemic encephalopathy: synthesis and meta-analysis of trial data BMJ 2010;340:c363.

How to cite this article:

Banashree Swain, Pradeep Kumar Jena and Mangal Charan Murmu (2020) 'Evaluation of Serum Neuron Specific Enolase and Other Biochemical Markers in Neonatal Hypoxic Ischemic Encephalopathy', *International Journal of Current Advanced Research,* 09(05), pp. 22103-22111. DOI: http://dx.doi.org/10.24327/ijcar.2020.22111.4356

****