Original Article

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Paediatrics Section

Lactate Dehydrogenase and Hepatic Transaminases as Prognostic Markers of Hypoxic Ischaemic Encephalopathy in Neonates with Perinatal Asphyxia

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ABSTRACT

Introduction: Hypoxic Ischaemic Encephalopathy (HIE) following perinatal asphyxia, due to cerebral hypoxia is an important cause of neonatal morbidly and mortality. Hypoxia causes cell death in various tissues like liver, kidney and muscles also. The leakage of enzymes from the dying cells causes elevation of Lactate Dehydrogenase (LDH), Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) etc.

Aim: To investigate whether serum LDH, ALT and AST levels in the first 12 hours of birth can be used as a predictor of severe HIE and/or the neurodevelopmental outcome at one year of age.

Materials and Methods: A prospective longitudinal diagnostic test evaluation was done on in neonates with perinatal asphyxia admitted in a tertiary level neonatal intensive care unit in South India from March 2014 to September 2015 (18 months). Blood was collected for assay of LDH, AST and ALT within the first 12 hours of life. They were staged using the Levene's modification of Sarnat and Sarnat into stage1, 2 and 3 (mild, moderate and severe) on day 3. The survived babies were assessed for the neurodevelopment using the Development Assessment Scale for Indian Infants (DASII) at 4, 8 and 12 months. Sensitivity, specificity, positive predictive value, negative predictive value and accuracy were calculated for each of these enzymes in predicting severe HIE and adverse neurodevelopmental outcome.

Results: Out of the 76 babies with perinatal asphyxia, 27 had mild HIE, 17 had moderate HIE, 16 had severe HIE and 6 babies died and 10 were not having HIE. On follow-up of 36 infants for a period of one year, 69% had normal development, 8% each had mild and moderate delay and 14% had severe delay in development. For LDH, a cut-off value of 92.8 IU/l and 1153IU/l had good sensitivity and specificity for severe HIE and mortality respectively. A cut-off value of 38.5 and 36 were obtained in case of ALT and AST in case of severe HIE; the values were 47.5 and 41.5, respectively in case of mortality. The values had good sensitivity, but specificity was found to be low. A cut-off value of 919 IU/l of LDH showed good sensitivity and specificity in predicting severe developmental delay.

Conclusion: Elevated LDH levels during first 12 hours of birth was found to be the best predictor of severe HIE, mortality as well as adverse neurodevelopmental outcome at one year of age in this study. AST and ALT levels also had significant predictive value in identifying severe HIE, but not in predicting outcome. These biochemical parameters are relatively inexpensive, easily available in most centers, can be used effectively to identify HIE early enough, as it is difficult to stage HIE if the baby is ventilated and on muscle relaxants and sedative antiseizure drugs, so that neuroprotective strategies like hypothermia can be started at the earliest.

Keywords: Alanine aminotransferase, Aspartate aminotransferase, Hypoxia, Neurodevelopmental asphyxia

INTRODUCTION

According to World Health Organisation (WHO) and Maternal and Child Epidemiology Estimation Group (MCEE) 2019, globally 2.5 million neonates die every year. Of these 24% is attributable to perinatal depression [1]. A significant proportion of survivors develop cerebral palsy, visual and hearing difficulties and cognitive impairment [2], as brain is the organ, most sensitive to hypoxia and ischaemia. APGAR score for the detection of neonatal asphyxia is non-specific, as it can be decreased during depression from maternal drugs, in babies with neuromuscular problems, trauma, or metabolic or infectious insults. Assessment of cord blood pH or Arterial Blood

Gas (ABG) is not available in most of the maternity centres. The scoring of HIE also becomes difficult in the ventilated babies, on muscle relaxants and antiseizure medications [3]. Thus cheap, easily available biochemical parameters become useful in suspected cases of HIE, to predict the severity and adverse neurodevelopmental outcomes.

Foetus exposed to hypoxia-ischaemia, centralises cardiac output to prioritised organs like the brain, heart and adrenals, at the expense of less important organs like the liver, lungs, skin and muscles, due to diving reflex [4]. This results in cell death leading to leakage of intracellular enzymes into the circulation. Aspartate aminotransferase is present in the liver, muscles, myocardium and RBC, Alanine aminotransferase in liver and LDH, in most of the body tissues. The elevated levels of these enzymes, persist for 5-36 hours in the serum, before they are cleared [4]. The estimation of these enzymes have been found useful in identification of HIE. It was aimed to assess any correlation between the serum levels of LDH, AST and ALT to the severity of HIE and/or adverse neurodevelopmental outcomes, which can help the neonatologist in early institution of neuroprotective strategies and in counseling regarding the chance for significant developmental delay or mortality.

MATERIALS AND METHODS

It was a prospective observational analytic study done in neonates with perinatal asphyxia admitted in a tertiary level NICU in South India from March 2014 to September 2015 (18 months). Institutional Ethical Clearance was obtained before recruiting the patients (IEC No 02/20/2014/MCT). Written informed consent from parents were taken before including the baby in the study.

Inclusion criteria: Neonates with gestational age >34 weeks with documented perinatal asphyxia indicated by any three of the following: Apgar score <6/10 at five minutes, umbilical cord pH/first blood gas pH <7.1, Meconium stained amniotic fluid and change in foetal heart rate.

Exclusion criteria: Neonates with confirmed central nervous system malformations, metabolic disorders, liver diseases, and chromosomal abnormalities, neonates born to mothers who would have received magnesium sulphate prior to delivery, TORCH infections and proven sepsis was excluded from the study.

The sample size was calculated based on a previous study [5], with a precision of 0.15, power of 80% and a p-value of less than 0.05, and is found to be 62. Assuming a dropout rate of 20 %, 76 children were selected.

The information regarding APGAR score, umbilical cord or first blood gas pH, detailed maternal history, meconium staining of amniotic fluid and immediate clinical parameters were collected from clinical records. The following investigation was done at admission: routine blood examination, sepsis screening, blood culture, random blood sugar, prothrombin time, International Normalized Ratio (INR), and ABG. The HIE markers like AST, ALT, and LDH were done before 12 hours of life. Hepatic transaminases were determined by IFCC (International Federation for Clinical Chemistry) method with accredited laboratory machines (Olympus AU400, Beckman Coulter Inc. USA). LDH was assayed by IFCC method on Cobas C systems (Roche diagnostics).

Other investigations like Electroencephalogram (EEG), Neurosonogram, Computerized Tomography (CT), Magnetic Resonance Imaging (MRI) and ECHO was done as per the discretion of the physician. The staging of HIE, according to Levene's modification of Sarnat and Sarnat [6,7] was done on the third postnatal day [Table/Fig-1]. Neurodevelopmental assessment was done by DASII (Developmental Assessment Scales for Indian Infants) [8] at four months, eight months and 12 months. DASII assessment was done by a single trained staff from the child development center attached to the hospital. Adverse neurodevelopment based on DASII is classified as severe, when the composite Developmental Quotient (DQ) is less than 70, moderate, when DQ is 70-85, and normal with a DQ of more than 85.

Stage 1 (Mild)	Stage 2 (Moderate)	Stage 3 (Severe)
Irritability, hyper alert	Lethargy	Comatose
Mild hypotonia	Moderate hypotonia	Severe hypotonia
Poor sucking	Require tube feeds	Failure to maintain spontaneous respiration
No seizure	Seizure	Prolonged seizure

[Table/Fig-1]: Levene's modification of Sarnat and Sarnat staging for HIE.

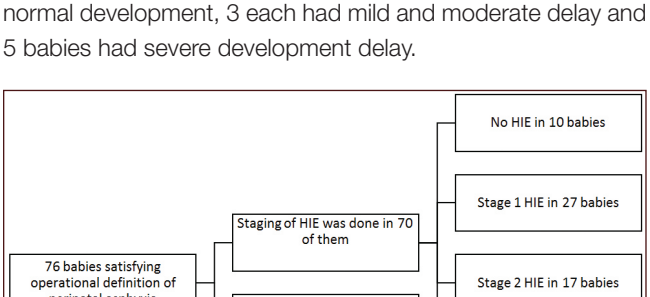
STATISTICAL ANALYSIS

Data were recorded using the Statistical Package for the Social Sciences (SPSS) 20.0 (Chicago, IL, USA). Diagnostic test evaluation was used in assessing whether LDH, ALT and AST predicts the severity of HIE. Sensitivity, specificity, positive predictive value, negative predictive value and accuracy were calculated for each of these enzymes in predicting severe HIE. A Receiver Operating Characteristic (ROC) curve analysis, where in the "state variable" was set to stage 3 HIE was performed to determine the cut-off values of LDH, ALT and AST levels using Youden index [9]. Accordingly, specificity and sensitivity were calculated. Similar analysis was also performed for predicting death in immediate newborn period and severe developmental delay at one year age. Student's t-test analysis was done for analyzing LDH, ALT and AST values with stage 3 HIE and death. A p-value <0.05 was considered statistically significant. Kappa coefficient was used to measure agreement between severe HIE and severe neurodevelopmental delay.

RESULTS

During the study period, there were 870 babies greater than 34 weeks of gestation admitted in the inborn nursery. Of these, 76 babies (8.7%) were enrolled after fulfilling the inclusion and exclusion criteria.

The schematic representation of the study is shown in [Table/Fig-2]. The clinical characteristics were shown in [Table/Fig-3]. Six babies died out of the 76; two babies that were initially classified as stage 2, became stage 3 after 48 hours. On follow-up for a period of one year, out of the 36 infants, 25 had normal development, 3 each had mild and moderate delay and 5 babies had severe development delay.



[Table/Fig-2]: Schematic diagram showing the staging of HIE in the present study.

Characteristics	Parameters	Number (%)
Gender	Male	46 (60.5%)
	Female	30 (39.5%)
Maturity	Term	57 (75%)
	Late preterm	19 (25%)
Birth weight	AGA (>2.5KG)	59 (77.7%)
	SGA (1.5 -2.5)	15 (19.7%)
	LGA >4kg	2 (2.6%)
Maternal age	<20 years	4 (5.26%)
	20_35 years	71 (93.42%)
	>35 years	1 (1.31%)
Parity	Multipara	22 (28.9%)
	Primi	54 (71.1%)
Mode of delivery	LSCS	31 (40.8%)
	Normal	39 (51.3%)
	Instrumental delivery	6 (7.9%)
Twin		6 (7.9%)
HIE	No	10 (13.16%)
	Stage I	27 (35.53%)
	Stage 2	17 (22.37%)
	Stage 3	16 (21.05%)

[Table/Fig-3]: Clinical characteristics.

The LDH, AST and ALT were significantly elevated in babies with severe HIE, compared to those without HIE and those with mild to moderate HIE. Babies with severe HIE showed a mean value of 1250 U/L, while with non-severe HIE, it was 333.85U/L. The mean value of LDH in the six babies who died, were 1379.5 with a standard deviation of 192.2. The sensitivity, specificity and positive predictive value of these enzymes in predicting severe HIE and mortality using ROC curves given in [Table/Fig-4,5], respectively. For LDH, a cut-off value of 92.8 IU/l and 1153IU/l had good sensitivity and specificity for severe HIE and mortality, respectively. A cut-off value of 38.5 and 36 were obtained in case of ALT and AST in case of severe HIE; the values were 47.5 and 41.5, respectively in case of mortality. The values had good sensitivity, but specificity was found to be low.

Prediction of severe HIE new born N=70					
Enzymes	**AUC**	**Cutoff**	**Sensitivity**	**Specificity**	**p-value**
LDH	0.993	92.8	94.4	100	0.008
ALT	0.944	38.5	100	75	0.027
AST	0.901	36.0	100	67.3	0.036

[Table/Fig-4]: Best cut-off values of enzyemes obtained of severe HIE from a receiver operating charcteristic curve (ROC) presented together with area under the curve (AUC).
LDH: Lactate dehydrogenase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; *p<0.05 statistically significant

Prediction of mortality n=6					
	AUC	**Cutoff**	**Sensitivity**	**Specificity**	**p-value**
LDH	0.956	1153.0	100	93.3	0.019
ALT	0.902	47.5	81.3	90	0.035
AST	0.907	41.5	100	75	0.034

[Table/Fig-5]: Best cut off values of enzyemes obtained of mortality from a Receiver Operating Charcteristic Curve (ROC) presented together with area under the curve (AUC).
LDH: Lactate Dehydrogenase; ALT: Alanine Aminotransferase; AST: Aspartate aminotransferase; <0.05 statistically significant

[Table/Fig-6] shows the cut-off values for LDH, AST and ALT for prediction of severe developmental delay at one year. For LDH, a cut-off value of 919 IU/l showed good sensitivity, and specificity in predicting the adverse outcome, cut-offs for ALT and AST did not reach statistical significance here.

DISCUSSION

This study clearly demonstrates that a cut-off value of 92.8 IU/l and 1153IU/l for LDH has good sensitivity, specificity and positive predictive value in identifying severe HIE and mortality in near term as well as term babies with perinatal asphyxia. Similar findings were observed in previous studies also [3,6]. It is interesting to note that two babies in the study, that were initially labelled as moderate HIE, turned out to be severe HIE, two days later. While one baby died following severe HIE, the

Prediction of severe neurodevelpmental outcome at one year of age N=36					
Enzymes	AUC	Cut-off	Sensitivity	Specificity	p-value
LDH	0.994	919	100	96.8	0.010
ALT	0.923	56	80	100	0.073
AST	0.884	43.5	80	87.1	0.089

[Table/Fig-6]: Best cut off values for enzyemes for prediction of severe neurodevelpmental ooutcme obtained from a Receiver Operating Charcteristic Curve (ROC) presented together with area under the curve (AUC).
LDH: Lactate Dehydrogenase; ALT: Alanine Aminotransferase; AST: Aspartate aminotransferase; <0.05 statistically significant

other one was followed-up till one year of age and had severe developmental delay as assessed by DASII. Both these babies, had LDH values more than 1200 U/l. This finding gives thrust to the fact that these enzymes measured in initial hours of birth, can predict the severity better than the staging of HIE, at least in a few cases. In severe HIE the sensitivity, specificity and cut-off values of ALT and AST are comparable with other studies [10-12]. This study had a higher cut-off compared to that of Karlsson M et al., [5]. It may be due to difference in the method of estimating ALT, or because of the variable patterns of organ damage in different populations.

Out of the 76 newborns with perinatal asphyxia, six babies died (21%) and five out of 36 babies that completed one year follow-up had severe developmental delay. This value is comparable to the study by Singh SK et al., from India [12]. LDH levels had higher sensitivity and specificity in predicting adverse neurodevelopmental outcome in the present study as against Karlsson M et al., [5]. ROC cut-off values for ALT and AST were found to have less statistical significance compared to LDH. The predictive value of these enzymes in infants, who died of HIE, has not been studied before. The predictive power of all the three enzymes were very good in respect of mortality [13]. It has been proven that neuroprotective strategies like hypothermia significantly improves the neurodevelopmental outcome of these babies [14]. Early identification of severe HIE by the assay of these enzymes, helps in timely institution of these strategies. This is especially useful in domiciliary deliveries, where details regarding perinatal insults often may not be available.

Limitation(s)

Out of the 60 survivors, only 36 babies (60%) could be followed-up for assessment of neurodevelopmental outcome at 4, 8 and 12 months, in spite of repeated reminders by post and telephone calls. The grading of HIE by Sarnat and Sarnat was considered as the gold standard in this study. However, MRI of brain done between second and tenth day of life is considered to be one of the best modality for diagnosing HIE and in predicting the neurological outcome. Most of the newborns enrolled in this study could not be subjected to MRI in the prescribed period due to logistic, technical and financial reasons in the government setting.

CONCLUSION(S)

This study reports that LDH, ALT and AST assay, done within the first 12 hours after birth are good predictors of severe HIE in term as well as near term newborns. These biochemical tests are relatively inexpensive and easily available in most centres. They can be used effectively to assess the severity of HIE earlier, so that neuroprotective measures like hypothermia can be instituted fast, especially when the history of perinatal depression is not available, or in ventilated babies on muscle relaxants. The LDH level has high sensitivity and specificity in predicting adverse neurodevelopmental outcome also, which can help in counselling the parents regarding outcome.

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