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ROLE OF SERUM BIOMARKERS IN MULTIORGAN DYSFUNCTION AND PREDICTING THE NEUROLOGICAL OUTCOME AND SEQUELAE IN NEONATES ≥ 36 WEEKS WITH BIRTH ASPHYXIA.

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ABSTRACT Hypoxic Ischemic Encephalopathy is a leading cause for mortality and morbidity in infants world wide. The estimated incidence of HIE is 1-2/ 1000 full term infants. Few studies claim even more. Based on severity of HIE, infants may end up in permanent disabilities and neurodevelopment al delays. Besides neuro compromise, multiorgan dysfunction like hepatic, renal, cardiovascular comorbities are often involved with HIE in infants. Early detection and reliable biomarkers can play a vital role in neuroprotection and to identify possible multi organ dysfunction.In this article we review biomarkers of HIE including S100B, Cystatin-C, Cardiac Troponin I and Lactate Dehydrogenase based on our own clinical study and other related studies. We hope to contribute to the awareness, validation, and clinical use of above Biomarkers.

KEYWORDS : Hypoxic Ischemic Encephalopathy, S-100 B, Cystatin-C, Cardiactroponin I and Lactate dehydrogenase.

INTRODUCTION

Biomarkers are specific molecules found in blood and other bodily fluids such as urine, CSF etc or tissues that are released in response to a sign of normal or abnormal physiological process, or any pathological insult of that specific organ. Early detection of Biomarkers and their reliability improves the prognosis of the condition and further line of treatment in infants with HIE. Clinically significant Biomarkers are key to alleviate morbidity and mortality. Serum Biomarkers such as S100B, Cystatin C, Cardiac Troponin I and Lactate Dehydrogenase can be used for the prognosis of specific comorbidity and severity of HIE and their correlation.In this study we compared our own clinical study and other related studies to the establish the significance of these biomarkers to create awareness and identifying gaps for further clinical guidance. A total of 84 infants have been enrolled and categorised accordingly depending on different grades of HIE based on NICHD assessment. All the four biomarkers were measured at baseline in patients belonging to all three grades of HIE, amongst which Serum protein S-100B, Cystatin-C, lactate dehydrogenase, cardiac troponin I were investigated in abnormal patients as assessed by MRI findings and Hammersmith neurological examination.The related articles were searched and included from electronic media from a combination of PubMed, MDPI, Research gate, Embase, and MEDLINE.

Biomarkers For Hypoxic-ischemic Enchphalopathy S100B

S100B protein is a calcium binding protein produced by glial cells. They are released in the body by glia in response to metabolic insult such as deprivation of oxygen , serum or glucose etc. S-100B is expressed and released by astrocytes. This secretion by glia is an early response to metabolic injury and is released in different body fluids through renal metabolism. The levels can be assessed by taking urine, cord blood, serum, CSF, umbilical arterial blood, peripheral arterial blood, saliva. S100B has a very low half life of about 1 -2 hours.

(Qiu Luo et al., 2019) studies its prognostic value to predict early brain damage recorded during the first 24 hrs of birth. The study shows Serum S100B at 24 hours had higher sensitivity, but its positive LR value was only 3.26, less than 10, and the negative LR value was 0.32, more than 0.1; thus, the statistical results showed a lower predictive value. The overall diagnostic sensitivity of serum S100B was 0.80 (95% confidence interval [CI] = 0.68-0.88) and the specificity was 0.79 (95% CI = 0.70-0.87). The study concluded increasedIncreased serum S100B level at 24 hours of postnatal life can demonstrate brain damage, but it should not be the only one used to predict PA outcome.

Another study by (Serafinna Perrone et al., 2023) on S100B as biomarker for early diagnosis and intervention of brain injury mentioned that sensitivity and specificity of S100B testing in urine and (serum monitoring time points up to 24 h) showing a sensitivity of 50% to 73% and a specificity of 74% to 90% in serum (Beharier's study). (Serafinna Perrone et al., 2023) stated S100 B relation in correlation with the severity of HIE. They mentioned the levels of S-100B levels were found to be high immediately after birth and continue to rise with time (up to one week) with severe asphyxiated newborns; in mild asphyxia, the blood levels exhibit only a slight elevation soon after birth and decline from this point further with time.Newborns with no signs of asphyxia, instead, present baseline levels of the protein at all times. They also stated most of the times S100B can be elevated in other conditions like Fetal Growth Restriction during pregnancies. S100 B can also play a role in fetuses who developed IVH after birth. This study concluded that s100B with combination of other biomarkers may improve the prognosis.

To bridge the gap of studying combination of biomarkers in our study we investigate the prognostic ability of Serum protein S-100B individually and in combination with other biomarkers of significant correlation with Serum protein S-100B. We enrolled 84 patients and were categorized in to different grades of HIE based on NICHD assessment. As observed from the study Serum protein S-100B exhibited high specificity (100.00) and sensitivity (76.47) values which makes them more reliable biomarkers for predictability of neurological events. LR+ 4.25, LR- 0.00 for S100B It was observed from ROC curve analysis that Serum protein S-100 B has a cut off value of >12. It can be inferred that these cut off values serve as prognostic biomarker concentrations that can predict the possibility of neurological sequelae in HIE infants. And further we studied Pearson correlation coefficient, it was observed that Serum protein S-100B had the strongest correlation with Cystatin-C amongst all the markers and a weaker correlation with Lactate dehydrogenase . Cystatin-C elevation has very strong correlation with Serum protein S-100B with R value of 0.084 and P value of <0.001.

Table 1 Pearson Correlation

Pearson Correlation	R value	P value
Cystatin –C vs Serum protein S-100B	0.840	<0.001**
Cystatin –C vs Lactate dehydrogenase	0.202	0.065+
Cystatin –C vs SGOT	0.483	<0.001**
Cystatin –C vs SGPT	0.785	<0.001**
Cystatin –C vs Blood urea(mg/dl)	0.680	<0.001**
Cystatin –C vs Serum Creatinine (mg/dl)	0.713	<0.001**
Cystatin –C vs PT1	0.285	0.009**

Based on the result of AUROC analysis (AUROC value of 0.958 for Serum protein S-100B and value of 0.972 for Cystatin-C) and because of high sensitivity and specificity values, Serum protein S-100B and Cystatin-C could possibly be two reliable biomarkers beyond the cut off values of which have prognostic value in predicting neurological sequelae. The observations are highly significant for both these biomarkers. These observations are backed by strong Pearson correlation seen amongst these two biomarkers.

Table 2 ROC Curve Analysis

Variables	ROC results to predict Advanced Stage				Cut-off	AUROC	SE	P value
	Sensitivity	Specificity	LR+	LR-				

Serum protein S-100B	100.00	76.47	4.25	0.00	>12	0.958	0.018	<0.001 **

Apart from understanding the predictability of these individual biomarkers, the combination of these biomarkers (S100B, Cystitin-C, Cardiac troponin I, have also been studied by ROC curve analysis A high sensitivity and specificity values of the combination of four markers suggest to check for the abnormalities of these biomarkers together in various grades of HIE. The high PPV (Positive predictive value) and high NPV (Negative predictive value) signifies a high degree of correlation between the combination of these four biomarkers and the predictability of neurological outcome such as HIE and other disorders.

Table 2 Combination Of Serum Protein S-100B, Cardiac Troponin I, Cystatin –C And Lactate Dehydrogenase To Predict The HIE 2 & 3

Combination of 4 markers	HIE1 (n=34)	HIE2&3 (n=50)	Total (n=84)
Negative	26(76.4%)	0	26(30.9%)
Positive (any one positive)	8(23.5%)	50(100.0%)	58(69.1%)

Sensitivity %	100.00
Specificity%	76.47
PPV%	86.21
NPV%	100.00
Accuracy%	90.48
Significance	c2=55.375; P<0.001**

Cystatin-C

Cystatin C is a cationic cysteine protease inhibitor which has low molecular weight produced by all nucleated cells in the body that is freely filtered by glomerulus and completely reabsorbed at proximal tubules. Many studies have suggested Cystatin C can be potential indicator for AKI (Acute Kidney injury) than Sr.Creatinine and Creatine clearance due to its rateof production which not affected by muscle mass, race , sex and diet unlike other indicators. Studies have shown decrease in GFR is associated with increase in Serum Cystatin-C thus signifying it as a better indicator for renal function and impairments.

Milena Treiber et al., (2014) in their study compared the predictive value of cystatin-C (cysC) and creatinine (Cr) in assessing acute kidney injury (AKI) following perinatal hypoxia/asphyxia. One hundred full-term newborns were examined, with 50 affected by hypoxia/asphyxia and 50 controls. CysC and Cr levels were measured in umbilical cord blood at birth (cysC-umb and Cr-umb) and in peripheral vein samples three days later (cysC-3 and Cr-3). Healthy newborns had cysC levels of 1.39 ± 0.19 mg/L at birth and 1.34 ± 0.21 mg/L after three days, showing no significant change. In the hypoxia/asphyxia group, cysC levels were significantly elevated in cord blood (2.12 ± 0.53 mg/L) and decreased by day 3 (1.56 ± 0.32 mg/L). Cr levels were also different at birth between control (62.74 ± 12.84 µmol/L) and hypoxia/asphyxia (72.60 ± 15.55 µmol/L) groups, declining significantly by day 3 in both groups. Receiver-operating characteristic analysis revealed cysC-umb as the most accurate diagnostic marker, with a chosen cutoff of 1.67 mg/L (sensitivity 84.0%, specificity 90.0%) or 1.69 mg/L (sensitivity 82.0%, specificity 94.0%). Overall, serum cysC emerged as a more sensitive indicator of glomerular filtration rate than Cr in hypoxic newborns.

Nafisa Hassan Refat et al., (2023) conducted a study aimed to evaluate the effectiveness of serum cystatin C (CystC) in predicting acute kidney injury (AKI) early in full-term neonates with perinatal asphyxia (PA), a condition that can lead to AKI. The research was conducted over two years at a neonatal intensive care unit. Among 70 full-term neonates with PA, 30% developed AKI. Those with AKI tended to have higher serum CystC levels (1.50 ± 0.12) compared to those without AKI (0.90 ± 0.14, $p < 0.001$). Low birth weight, serum CystC levels, hypotension, and severe hypoxic-ischemic encephalopathy (HIE) were predictive factors for AKI. Serum CystC demonstrated a 94.3% accuracy in predicting AKI. The study concluded AKI is prevalent in neonates with PA, and serum CystC shows promise as an early biomarker for AKI prediction in such cases, but further studies are needed for confirmation.

Our observational study investigated the correlation between Cystatin C and its association with hypoxic-ischemic encephalopathy (HIE) in predicting neurological outcomes, survival with sequelae, at 14 days or later post-birth asphyxia, and at three months of age. We also compared serum biomarkers with the National Institute of Child Health and Human Development (NICHD) score for predicting neurological outcomes. The study involved 84 patients categorized into different HIE grades based on NICHD assessment. Baseline measurements of Serum Creatinine, Serum Urea, and Serum Cystatin-C were taken across HIE grades with Echo findings and neurological examinations. Elevated Serum Cystatin-C levels (>1.32) at 14 days and three months exhibited sensitivity (92.00), specificity (100.00), and an ROC value of 0.972, serving as an independent prognostic marker for abnormal neurological outcomes. Infants with abnormal echo findings demonstrated a significant 95% increase in Serum Cystatin-C levels after 72 hours, indicating its potential as a prognostic biomarker. The study highlighted clinically significant increases in Serum Cystatin-C levels among severe HIE cases, progressively after 72 hours, at 14 days (48% increase), and at three months (116% increase). Serum Creatinine and Blood Urea concentrations were also notably important in severe HIE subjects.

Table 3 ROC Curve Analysis Values For Cystatin-C , Sr. Creatinine

Variables	ROC results to predict Advanced Stage				Cut-off	AUROC	SE	P value
	Sensitivity	Specificity	LR+	LR-				
Cystatin-C	1.26 ± 0.04	1.86 ± 0.38	1.98 ± 0.41	1.30 ± 0.10	1.72 ± 0.26	2.37 ± 0.51	1.23 ± 0.04	1.74 ± 0.28
Blood urea (mg/dl)	86.00	100.00	0.00	0.14	>27	0.964	0.0168	<0.001**
Serum Creatinine (mg/dl)	88.00	100.00	0.00	0.12	>0.8	0.981	0.0108	<0.001**

Cardiac Troponin- I

Cardiac Troponin I levels in blood will be very low for the new borns (<30 pg/mL). But incase of any cardiac injuries or Myocardial Ischemia the levels can significantly increase. Early elevation of Cardiac troponin I in asphyxiated neonates is a predictor for Myocardial dysfunction. Presence of Echo findings with early elevation in levels of Cardiac troponin -I correlates with Myocardial dysfunction.Aravind Shastry et al.,(2012) performed a retrospective study on the correlation of Cardiactrponin I and Clinical grade of HIE with in 36 hrs of birth. And the study inferred median concentrations of Cardiac troponin I increase with severity of HIE. Median (95% CI) cTnI concentrations were 0.04 µg/L (0.02–0.07 µg/L) in grade 1 HIE, 0.12 µg/L (0.08– 0.20 µg/L) in grade 2 HIE and 0.67 µg/L (0.41–1.35 µg/L) in grade 3 HIE. So as the inotropic support the higher the grade of severity the longer the inotropic support. The study supported the use of Cardiac Troponin I as a biomarker for anticipating the severity of mayocardial infraction in asphyxiated neonates.

Another study from Montaldo et al., (2014) demonstrated Creatinie Kinase MB and Cardiac Troponin I (cTnI) can be used to predict neurodevelopment outcome at 18 months in infants with perinatal asphyxia (PA) and to assess its diagnostic value in myocardial dysfunction.In their retrospective study with 178 neonates with PA. The results show significant correlation between BSID-II scroing and Cardiactropinin I concentration with mental development index (r-0.69, P< 0.05) and psychomotor development index (r-0.39, P<0.05). Serum cTnI concentrations and duration of inotropic support were significantly greater with increasing severity of PA. cTnI was negatively correlated with fraction shortening (r −0.64; P<0.05). The severity of tricuspid regurgitation was correlated with the cTnI concentration (r 0.61; P<0.05). The study concludes that cardiac Troponin I concentrations correlates with the severity of HIE , myocardial dysfunction and with Bailey II at 18 months.

Our clinical study explores Cardiac Troponin I as a potential biomarker for Myocardial Dysfunction and neurological outcomes in neonatal HIE. Significant direct relationships were observed between Cardiac Troponin-I levels and HIE grades, establishing its prognostic value for neurological sequelae prediction. Higher HIE grades correlated with elevated Cardiac Troponin I concentrations. Neonates with abnormal echo findings displayed a 50% higher Cardiac Troponin I upon admission and at 24 hours than normal infants, with a consistent 25% increase at 72 hours, indicating Myocardial dysfunction presence. Early elevation of Cardiac Troponin I suggests Myocardial dysfunction, with its progressive increase indicating worsening HIE

severity. Elevated Cardiac Troponin levels (25%-75% increases) in neonates and infants with abnormal conditions, beyond the cutoff value (> 0.042), predicted neurological sequelae. Cardiac Troponin I emerged as a moderately independent biomarker via AUROC analysis. Further investigation into Myocardial dysfunction's relationship with HIE severity relative to Cardiac Troponin I and Cardiac Troponin T levels could provide crucial clinical insights.

Table 4 ROC Curve Analysis Of Cardiac Troponin-I

Variables	ROC results to predict Advanced Stage				Cut-off	AUROC	SE	P value
	Sensitivity	Specificity	LR+	LR-				
Cardiac troponin I	58.00	100.00	0.00	0.42	>0.042	0.722	0.056	<0.001**

LDH

As liver is the site for various metabolic activities, when there is an ischemic condition due to hypoxia it may lead to increase in different hepatic enzymes like Lactate Dehydrogenase(LDH), Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT), activated partial thromboplastin time (aPTT),Prothrombin Time Test (PT) indicating liver dysfunction[4] As newborns are often assessed outside of tertiary-care centres, easily accessible biomarkers like liver enzymes are useful for an early diagnosis and prediction of outcomes. These markers will help in identifying infants that are more suitable for intervention in the first few hours of life.

Rajeesha et al. studied enzyme levels (LDH, AST, ALT) in 30 asphyxiated neonates at different times post-birth. They aimed to assess enzyme correlations with asphyxia severity and determine optimal testing windows. LDH levels increased over time, peaking at 18-24 hours (2153.10 ± 1992.15 U/L). Strong correlations were found between LDH within 4 hours and at 18-24 hours, particularly with severe HIE. Best prediction time for asphyxia severity using LDH and AST was 18-24 hours post-birth. This study underscores the importance of early biomarker assessment, highlighting accurate windows for LDH and AST in birth asphyxia severity prediction. Higher cord blood LDH indicated worse asphyxia, while ALT levels didn't significantly differ within 24 hours.

Rajiv et al., (2021) investigated the impact of birth asphyxia on liver function at the cellular level, leading to the release of liver enzymes into the bloodstream and altered liver function in case control study involving 115 cases (full-term neonates with APGAR scores of 6 or less at 1 minute) and 115 controls (neonates with APGAR scores of 7 or more) The goal was to examine these changes in hepatic function in relation to different stages of hypoxic ischemic encephalopathy (HIE).Blood samples were collected 72 hours after birth, and liver function tests were performed to compare the two groups.The results showed statistically significant differences in the levels of aspartate transferase (AST), alanine transferase (ALT), lactate dehydrogenase (LDH), and alkaline phosphatase (ALP) between the cases and controls ($p<0.05$). Additionally, the study found that the differences in AST, ALT, and LDH levels were statistically significant between HIE stage 1 and stage 2 ($p<0.5$), as well as between HIE stage 1 and stage 3 ($p<0.5$), but not significant between HIE stage 2 and stage 3 ($p>0.5$) The study demonstrated that full-term neonates with perinatal asphyxia had higher serum levels of hepatic enzymes compared to those without asphyxia, specifically at the 72-hour mark. Moreover, the levels of these enzymes increased with the severity of HIE

In our prospective cohort study, we investigated liver dysfunction's correlation with different HIE grades. HIE assessment utilized the NICHD score, while MRI and spectroscopy predicted brain injury outcomes. MRI showed a 100% positive predictive value in detecting brain injury. Liver enzymes (LDH, SGOT, SGPT, aPTT, PT) were compared across HIE grades upon admission, at 24 and 72 hours post-admission in asphyxiated neonates. Correlation between LDH and MRI findings predicted outcomes. Our study revealed significant hepatic enzyme elevation (LDH, SGOT, SGPT, aPTT) over 72 hours in all HIE grades, indicating hepatic dysfunction's presence and extent. Liver biomarker levels (LDH, SGOT, SGPT, aPTT) increased proportionally with HIE severity, making them clinically valuable for liver assessment in birth asphyxia. LDH's potential as a prognostic biomarker for neurological sequelae from HIE was explored through MRI and Hammersmith examination correlation in normal and abnormal subjects. Serum LDH concentrations were higher in subjects with abnormal MRI changes and neurological examination results at 14 days and 3 months. ROC analysis suggested LDH lacked independent prognostic capability due to a low AUROC value (0.573) and non-significant p-value.

Table 5 ROC Curve Analysis Of Liver Biomarkers

Variables	ROC results to predict Advanced Stage				Cut- off	AUROC	SE	P value
	Sensitivity	Specificity	LR+	LR-				
Lactate dehydrogenase	234.79 ±8.35	290.73 ±135.99	617.23 ±133.47	318.74 ± 69.56	444.32 ± 80.09	762.62 ± 103.02	327.32± 51.12	594.14 ±92.56
SGOT	86.00	100.00	0.0	0.14	>112	0.880	0.046	<0.001**
SGPT	92.00	100.00	0.0	0.080	>42	0.978	0.012	<0.001**
APTT	26.00	100.00	0.0	0.74	>29	0.505	0.061	0.938
PT	100.00	35.29	1.55	0.00	>11	0.644	0.060	0.017*

Over View Of Materials And Methods Of The Present Study

Study Design And Setting

This was an observational correlational clinical study carried on from September 2016 to march 2017 at Department of neonatology, Madras Medical College (MMC), Chennai. Also involved are out-born unit of Institute of Child health and hospital for children (part of MMC) and inborn unit of Institute of Obstetrics and Gynaecology, Chennai (part of MMC). A written consent of willingness for enrolment of the infants in the study was obtained from the parents / care-givers of the infants selected. All 84 infants selected belonged to any of the three stages of Hypoxemic ischemic encephalopathy (HIE).

Eligibility

All new borns admitted in the intramural and extramural, with perinatal asphyxia were eligible for this study

Inclusion Criteria

Neonates born at ≥36 weeks of gestation and of >1800 g birth-weight, and with perinatal asphyxia were eligible. Perinatal asphyxia in patients born at the study hospital was defined as the need for resuscitation at birth, along with the presence of one or more of the following:

1. Apgar score of <6 – at 5 min after birth,
2. Continued need for resuscitation, for > 5 mins
3. umbilical cord pH or any arterial pH of <7.00 within 60 mins of birth, and base deficit of >16 mmol/L within 60 mins of birth

Written consent was obtained from the parents/caregivers of the infants selected for willingness for enrolment in the study (Annexure I).

Exclusion Criteria

1. Neonates with major congenital anomalies
2. Neonates presenting after 24 hours of birth
3. Neonates enrolled in the therapeutic Cooling Study

Primary outcome measure is to study the role of Serum Lactate dehydrogenase in assessing live dysfunction along with other liver enzymes in Perinatal asphyxia subjects ans its role in prediction of neurological sequelae.

Non-Parametric Setting For Qualitative Data Analysis

Pearson correlation between study variables is performed to find the degree of relationship, Pearson correlation co-efficient ranging between -1 (weak correlation) to 1 (strong correlation) between two biomarkers. The stronger the correlation, stronger is the ability to influence the other biomarker with which there is a correlation.

Statistical Methods

Descriptive and inferential statistical analysis has been carried out in the present study. Results on continuous measurements are presented on Mean+ SD (Min-Max) and results on categorical measurements are presented in Number (%). Significance is assessed at 5 % level of significance. The following assumptions on data are made

1. Dependent variables should be normally distributed,
2. Samples drawn from the population should be random,
3. Cases of the samples should be independent

Analysis of variance (ANOVA) has been used to find the significance of study parameters between three or more groups of patients, 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) on metric parameters. Chi-square/ Fisher Exact test has been used to find the significance of study parameters on categorical scale between two or more groups.

Statistical Software

The Statistical software namely SAS 9.2, SPSS 15.0, Stata 10.1, MedCalc 9.0.1 ,Systat 12.0 and R environment ver.2.11.1 were used for the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables etc.

RESULTS AND DISCUSSION

84 infants with a gestation age of > 36 weeks of gestation were included in the study. 19% (16/84) of infants were delivered through LSCS mode of delivery while the remaining infants delivered through normal route. 90% (76/84) of infants are of birthweight 2.5kg-3.5kg and 79 infants were AGA. Respiratory support was given 79 infants.

Demographic Data Of The Patients

Patients enrolled are of both genders – 35 male and 49 female. 39/84 patients were of gestational age less than 38 weeks. Except 8 neonates who are below 2500g, remaining 76 neonates enrolled have a birth weight between >2500g and 3500g. Only five were small for gestational age. 79 neonates required respiratory support on admission (CPAP or mechanical ventilation or O2). Oxygen saturation was still less than 90 in case of 42 neonates upon admission and in 76 neonates mean arterial pressure upon admission is <30 mm to 40 mm of Hg. Upon admission, patients were categorised based on the severity of encephalopathy – 34 patients were assessed mild (HIE1), 37 were assessed moderate (HIE2) and 13 were assessed severe (HIE3). All three grades of encephalopathy are identified among neonates upon admission – 34 neonates with mild encephalopathy (HIE1), 37 neonates with moderate encephalopathy (HIE2), 13 neonates with severe encephalopathy (HIE3). All patients were assessed with APGAR score at 1 minute, as <7 and at 5 minutes the score is <7 in 28 patients. Around 54% of patients in both HIE2 and HIE3 groups still have APGAR score <7 at 5 min. The demographic data is summarised in Table 6.

Table 6 Demographic Data

	Final Diagnosis			P value
	HIE 1 (n=34)	HIE 2 (n=37)	HIE 3 (n=13)	
Gender				
• Female	24(70.6%)	19(51.4%)	6(46.2%)	0.162
• Male	10(29.4%)	18(48.6%)	7(53.8%)	
Gestation age (weeks)				
• 36-37	19(55.9%)	12(32.4%)	8(61.5%)	0.070+
• 38-40	15(44.1%)	25(67.6%)	5(38.5%)	
Birth weight (g.)				
• <2500	7(20.6%)	1(2.7%)	0(0%)	<0.001**
• 2500-3000	21(61.8%)	17(45.9%)	9(69.2%)	
• 3000-3500	6(17.6%)	19(51.4%)	4(30.8%)	
Mode of delivery				
• LSCS	0(0%)	11(29.7%)	5(38.5%)	
• Vaginal	34(100%)	26(70.3%)	8(61.5%)	<0.001**
APGAR score 1 min.				
• <7	34(100%)	37(100%)	13(100%)	1.000
• >7	0(0%)	0(0%)	0(0%)	
APGAR score 5 min.				
• <7	1(2.9%)	20(54.1%)	7(53.8%)	<0.001**
• >7	33(97.1%)	17(45.9%)	6(46.2%)	
Cord ABG (pH, Base deficit)				
• <6.8	0(0%)	0(0%)	0(0%)	<0.001**
• 6.8-7.2	20(58.8%)	26(70.3%)	13(100%)	
• >7.2	14(41.2%)	11(29.7%)	0(0%)	
Severity of encephalopathy @ admission (NICHD assessment)				
Mild	34(100%)	0(0%)	0(0%)	<0.001**
Moderate	0(0%)	37(100%)	0(0%)	
Severe	0(0%)	0(0%)	13(100%)	

** Chi-Square test/Fisher Exact test

At base lines, blood samples were taken upon admission and after 24 hrs and 72 hrs to analyse for various prospective prognostic biomarkers such as Lactate degydrogenase, SGOT, SGPT, aPTT , PT.

MRI findings and Hammersmith neurological examination have been done. Baseline investigations of biomarkers are presented in table 2.

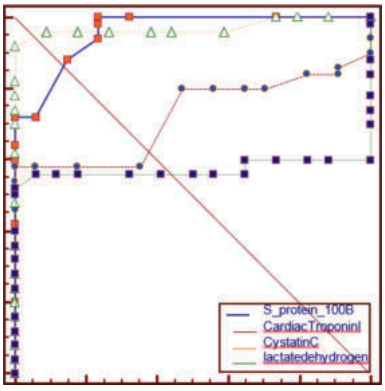
Table 7: Baseline Investigations Of Serum Biomarkers

Marker	Upon admission**			24 hrs after admission**			72 hrs after admission**		
	HIE 1	HIE 2	HIE 3	HIE 1	HIE 2	HIE 3	HIE 1	HIE 2	HIE 3
Serum protein S-100B	11.50 ±1.50	19.57 ±5.24	23.69 ±1.03	10.09 ±1.22	17.07 ±2.57	22.62 ±2.33	9.35 ±1.74	11.57 ±2.10	16.77 ±2.17
Cystatin-C	1.26 ±0.04	1.86 ±0.38	1.98 ±0.41	1.30 ±0.10	1.72 ±0.26	2.37 ±0.51	1.23 ±0.04	1.74 ±0.28	5.70 ±8.12
Cardiac troponin I	0.04 ±0.00	0.05 ±0.01	0.07 ±0.01	0.04 ±0.00	0.06 ±0.02	0.08 ±0.01	0.04 ±0.00	0.04 ±0.00	0.07 ±0.02
Lactatedehydrogenase	234.79 ±8.35	290.73 ±135.99	617.23 ±133.47	318.74 ±69.56	444.32 ±80.09	762.62 ±103.02	327.32 ±51.12	594.14 ±92.56	828.00 ±60.43
SGOT	107.32 +2.66	145.97 +47.21	215.38 +34.75	90.97 +8.86	186.97 +67.42	269.38 +28.50	74.38 +5.41	179.27 +76.29	300.62 +45.47
SGPT	39.00 +1.87	87.03 +30.24	86.00 +5.00	50.65 +18.16	88.27 +24.63	112.31 +13.38	47.47 +22.82	76.70 +17.83	152.23 +20.48
Blood urea (mg/dL)	22.91 +2.45	46.54 +21.63	44.92 +3.45	27.53 +3.15	56.05 +21.74	53.62 +3.50	21.35 +3.72	57.46 +21.79	76.38 +2.53
Serum Creatinine (mg/dL)	0.49 ±0.16	1.04 ±0.20	1.56 ±0.31	0.75 ±0.11	1.32 ±0.14	1.80 ±0.50	0.84 ±0.09	1.54 ±0.35	2.29 ±0.41

APTT	27.06 ±1.43	26.11 ±1.51	37.69 ±3.47	26.35 ±1.54	30.05 ±2.69	41.62 ±1.80	30.71 ±1.06	32.76 ±1.79	38.38 ±6.81
Blood sugar	81.35 ±6.49*	73.97 ±18.32*	69.69 ±19.52*	90.91 ±2.22	75.68 ±23.60	64.15 ±14.5	81.62 ±6.36	73.27 ±19.55	55.54 ±17.27

Table 8 ROC Curve Analysis Of Four Biomarkers Of Interest And Graphical Representation.

Variables	ROC results to predict Advanced Stage				Cut off	AUROC	SE	P value
	Sensitivity	Specifivity	LR+	LR-				
Cystatin –C	92.00	100.00	0.0	0.08	>1.32	0.972	0.0188	<0.001**
Serum protein S-100 B	100.00	76.47	4.25	0.00	>12.0	0.958	0.0180	<0.001**
Cardiac troponin I	58.00	100.00	0.0	0.42	>0.042	0.722	0.056	<0.001**
Lactate dehydrogenase	52.00	100.00	0.0	0.48	>250	0.573	0.069	0.292



Future Directions

More studies on different combination of biomarkers and emphasis on early detection with in first few hours of life can bring a better understanding HIE and suspected neurological outcomes. Further research on biomarkers which are least invasively available can promote early detection as most of the biomarkers will peak their concentration in first few early hours of life (6 hrs- 12 hrs). Further studies on combination of neurobiomarkers alone can give us valuable prognosis on the specific areas of brain that are or can be affected as a result of neurological seaqulae. Studies on clinically Significant and reliable biomarkers either single or in combination can help clinicians to take quick decision on proceeding to Hypothermia which is promising and emerging treatment for neuroprotetion in HIE infants and to prescribe neuroprotective agents in bedside care. Apart from neuroprotection Multiorgan dysfunction is so much prevalent in all HIE infants. Further more research on combination of biomarkers, least invasive procedures for obtaining samples and taking samples at accurate time i.,e during the peak of release of biomarkers before their half-life will be useful in treating HIE infants with anticipated multiorgan dysfunction and neurological sequeale.In the future, more sensitive and precise instruments for brain imaging (such as brain MRI), brain functioning (such as NIRS, aEEG), and long-term neuro-assessment should be incorporated to validation of biomarkers of neonatal brain injury.

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