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Serum S100b: Biochemical marker of brain damage in newborns with hypoxic ischemic encephalopathy

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ABSTRACT

Background. Hypoxic ischemic encephalopathy (HIE) is a major cause of neurological disabilities in infants. Several possible early biomarkers for hypoxic injury have been investigated but none of them have been completely utilized in clinical practice. Serum S100b is one such early biomarker.

Objective. The above study aims to assess the usefulness of the biomarker S100b in predicting the severity as well as the outcome of neonates having HIE.

Materials and methods. A prospective cohort study was done on 80 term newborns admitted to the neonatal intensive care unit (NICU), Institute of medical sciences & SUM hospital, Bhubaneswar from August 2019 to February 2021. Out of the total 80 term newborns, 20 newborns without any evidence of perinatal hypoxia were categorized as the Controls, and the rest of 60 newborns with perinatal hypoxia were categorized as Cases. Cases were further divided into 3 subgroups based on Sarnat and Sarnat staging with 27 newborns in HIE I, 17 newborns in HIE II, and 16 newborns in HIE III. Serum S100b was measured in all the above children at 4 and 48 hours of life.

Result. In our study, the mean serum S100b level at 4h & 48h of life was 1.15 & 1.05 respectively in controls and 3.24 & 2.36 (p<o.oo1) respectively in cases. The mean S100b level in subgroup HIE I, II, III were 2.06, 2.79 & 5.12 at 4h of life and 1.35, 1.86 & 4.08 at 48h of life, which is gradually increasing with the severity of asphyxia. On follow-up, it was found that babies with high S100b at 4h & 48 h of birth had more neurological sequelae. ROC curve depicted area under the curve was 0.80 showing high predictability of S100b for the neurological outcome. It was also seen that the cut-off value of 3.09 at 4 h has a sensitivity of 80% and specificity of 78.2%.

Conclusion. Serum S100b is not only an early biomarker for HIE but also helps in prognostication, which is an essential part of the treatment of newborns and during the counseling of parents.

Keywords: S 100b, perinatal asphyxia, hypoxic-ischemic encephalopathy

INTRODUCTION

Hypoxic-ischemic encephalopathy (HIE) resulting from perinatal asphyxia remains the major reason for neonatal morbidity and mortality worldwide [1]. HIE has an incidence of 1 to 3 per 1000 in term infants and is seen in around 60 percent of premature newborns [2]. Around 15-20% of affected newborns die in the postnatal period and around 25 percent develop permanent and severe neuropsychological disabilities like mental retardation, cerebral palsy, and learning disabilities [3,4] . The ability of clinicians to predict the final outcome of neonates suffering from HIE is a difficult task. The grading system of Sarnat & Sarnat score has defined the various stages of HIE based on clinical criteria. The categories in this scoring system are mild, moderate, and severe [5]. It also assesses the progression of the neurological insult to the neonatal brain and thus the prognosis [4]. But this scoring system is quite subjective and changes periodically. Amplitude integrated electroencephalogram (aEEG), is a new tool that may help stage the severity of the neurological injury as well as predict the prognosis [6]. Nevertheless, neither

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Sarnat & Sarnat scoring nor aEEG is effective in predicting the outcome in HIE neonates during hypothermia. They also do not give any information regarding the possible timing of brain injury. MRI brain helps in determining the timing and degree of injury, but obtaining an MRI is not very easy in criticalpatients.

The primary goal of a biomarker is to determine the time of injury and to predict long-termoutcomes. This is especially important in the case of hypoxia as it often begins in utero. S100b is a calcium-binding protein. It is a major component of cytosol in different cells which regulates various intracellular processes such as cell cycle regulation, cell growth, transcription as well as differentiation. At physiologic concentrations, it has a neurotrophic effect during both development and nerve regeneration. The glial cells in particular have a high concentration of S100b. Various immunoassay kits of S100b are commercially available and can be done in various body fluids like blood, urine, amniotic fluid, CSF, and saliva soon after birth [7]. Furthermore, reference ranges are available for both term and preterm healthy newborns. So S100b measurements in asphyxiated babies from umbilical artery blood will be helpful in the early diagnosis and grading of HIE and in predicting the outcome with reasonable sensitivity and specificity [8,9]. Thus, helping in early intervention with neuroprotective therapies like hypothermia and other neuroprotective agents which are emerging treatments for HIE [10].

Identification of good serum biomarkers that can facilitate clinical decisions is very essential as HIE is a rapidly progressing disease that can cause severe neurological sequelae. So, the above case-control study is done with an aim to assess the usefulness of S100b in hypoxic-ischemic encephalopathy (HIE) neonates, as a biomarker in predicting the severity and outcome.

MATERIAL AND METHODS

This case-control study was conducted in the Institute of Medical Sciences & SUM Hospital, Bhubaneswar which is a tertiary care hospital in the eastern part of India, for over 1 year 6 months (August 2019 to February 2021) after getting approval from the Institutional Ethical Committee. Informed consent was taken from the legally acceptable representatives.

Study population

80 neonates were recruited in the study that including controls and cases. We included all the term neonates (≥ 37 completed gestational weeks) of either sex, both inborn and outborn (those who reached the hospital within 4 h of birth) irrespective of their birth weight in our study. The diagnosis of perinatal asphyxia was made on clinical signs during the early hours of life along with perinatal arterial blood pH (i.e., umbilical artery or peripheral artery) and base deficit along with obstetric data for signs of fetal distress. For outborn babies in whom umbilical arterial blood gas was missing, peripheral arterial blood gas was done immediately on admission.

Cases

We included neonates fulfilling either of the following inclusion criteria: (i) APGAR score <7 at 5 mins, (ii) Positive pressure ventilation (PPV) for > 3mins and or pathological Cardiotocograph (CTG) (2 or more features which are non-reassuring or any abnormal features), (iii) newborns with clinical signs of perinatal asphyxia with perinatal arterial pH <7.1 and Base deficit >12 mmol/L.

Controls

Healthy term inborn newborns with no history of perinatal asphyxia and normal CTG.

Newborns with severe sepsis, congenital anomalies, and congenital neuromuscular disorders were excluded from the study.

After recruitment of cases and initial stabilization, the neurological assessment was done at regular intervals during the first 6 hours of life using Sarnat & Sarnat scoring by the experts (who were blinded to the biochemical results) and was eventually classified into HIE I, HIE II, HIE III based on the highest score during this period. Very few studies were available where the serum levels of S100b were measured. Considering the values of previously available studies and taking alpha error to be 5%, the power of the study at 80% with a 95% confidence interval the sample size was calculated.

Neurological assessment

Neurological assessment was done using the development assessment scale for Indian infants (DASII) score which is an Indian adaptation of the Bayley scales of infant development. It contains motor and mental scales. The assessment was carried out by a single trained examiner at 1,3,6,9 and 12 months of life for all the newborns included in this study and came for follow-up. The neurological outcome was defined at 1 year of age. Developmental delay is defined as “developmental quotient (DQ) ≤70 (2SD) in either the motor or mental scale”.

Blood sampling and testing

Newborns born between August 2019 to January 2020 were recruited as our study population. Sample collection for both the cases and controls was done

over 6 months. The newborn blood samples as required for Electrochemiluminescence (ECLIA) were obtained at 4 h and 48 h of life. Samples were collected by aseptic methods and were transferred to the laboratory on the same day maintaining a proper temperature. Blood was collected in EDTA vials and was processed within 4 hours of collection. None of the blood samples in the control group were hemolysed, whereas 8 samples from the cases group (1, 3, and 4 in HIE I, HIE II, and HIE III respectively) were hemolysed and hence were excluded from the study. Between collection and processing, the vials were stored at 2–8 degrees centigrade. A 50 µL blood sample was added to a biotinylated monoclonal S100b- specific antibody and a monoclonal S100b-specific antibody labeled with ruthenium complex and was incubated for 9 minutes. A sandwich complex was formed with S100b consisting of a biotinylated and a ruthenylated antibody. Streptavidin-coated microparticles were added to this complex and incubated for 9 minutes resulting in the formation of a solid-phase complex with the interaction of streptavidin and biotin. This reaction mixture was aspirated into a measuring cell where microparticles were captured magnetically on the surface of the electrode. Unbound substances were removed and voltage was passed through an electrode which induced a chemiluminescent emission that was measured by a photomultiplier.

TABLE 1. Demographic data and baseline characteristics of the study population (n=80)

	Controls (n = 20)	HIE I (n=27)	HIE II (n=17)	HIE III (n=16)
Age of Mother (y)	26.3 ± 3.7	27.4 ± 4.1	27.2 ± 3.1	27.6 ± 3.7
Parity (P1/P2/ P3/P4)	9/6/4/1	8/6/4/1	8/7/1/1	7/4/5/0
Inborn/ Outborn	20/0	22/5	14/3	6/10
Mode Of Delivery (NVD/Assisted/ LSCS)	10/3/7	10/6/11	5/3/9	3/2/11
Gestational age at birth (wk)	38.2 ± 1.2	38.3 ± 1.2	38.4 ± 1.2	38 ± 1.5
Birth Weight (Gms)	2767 ± 300	2821 ± 276	2477 ± 388	2377 ± 393
Gender (M/F)	11/9	15/12	8/9	9/7
MedianAPGAR At 5 Mins	8 (6-9)	6 (5-7)	5 (3-6)	4 (2-5)
CTG (Normal/ Pathological)	20/0	8/16	3/11	0/9
Perinatal arterial pH	7.18 ± 0.08 (7.03-7.33)	7.02 ± 0.07 (6.80-7.15)	6.91 ± 0.12 (6.60 -7.14)	6.78 ± 0.17 (6.53-7.10)
Perinatal arterial base deficit	11.2 ± 2.29 (7.1-15.9)	15.8 ± 3.4 (6.9-24.1)	18.04 ± 4.20 (9.4-28.4)	23.13 ± 4.15 (11.7-29.3)
Non-Invasive (CPAP/NIV)	5	10	9	3
Mechanical Ventilation	0	0	5	13

**NVD: Normal vaginal delivery, LSCS: Lower segment cesarean section, CTG: Cardiotocograph,*
CPAP: Continous positive airway pressure, NIV: Non-invasive Ventilation.
***Median APGAR scores were taken in all the newborns.*

All the controls were inborn, but 18 cases were outborn babies (5, 3, 10 from HIE I, HIE II, HIE III) in whom umbilical arterial blood gas data were missing, so peripheral arterial blood gas was done immediately on admission. APGAR score at 5 mins was missing in 10 patients and CTG was missing in 13 patients (3,3,7 from HIE I, HIE II, HIE III). None of the controls and HIE I baby required invasive mechanical support, whereas 18 babies were ventilated..

Statistical analysis

The data collected was entered in Microsoft excel 2019 and was analyzed using the SPSS software version 21. The quantitative data were expressed by mean and standard deviation. Differences in the mean between the same groups were determined by paired sample t-test or Wilcoxson signed-rank test and between two independent groups were calculated using unpaired sample t-test or Mann – Whitney test. Differences in mean in more than two groups were calculated using ANOVA or Kruskal-Wallis test. The qualitative data were expressed in percentages and the differences between percentages were computed using the Chi-square test or Fischer exact test. The receiver operator characteristic (ROC) curve was used to determine the cut-off level of serum S100b for the outcome, specificity and sensitivity. P-value< 0.05 was considered statistically significant.

RESULTS

A total of 20 healthy controls and 60 cases were recruited over 6 months. Out of 60 cases, 27 were classified as HIE I, 17 as HIE II, and 16 as HIE III. Among the cases 32 were male and 28 are female. The mean age group of mothers of the newborn was 27.1 ± 3.7 years and ranges from 20 to 35 years. The mean birth weight of the study subjects was 2626 ± 381 grams. Table 1 shows the clinical and demographic data of the study population.

Table 2: The blood samples were collected at 4 and 48 h of life. None of the control samples were hemolysed but 8 samples from cases were hemolysed (1,3,4 sample from HIE I, HIE II, HIE III respectively). So, these samples were not processed and were excluded from the study. Accordingly, 52 case samples (26, 14, 12 from HIE I, HIE II, HIE III respectively) and 20 control samples were included in the final analysis. All

TABLE 2. Mean S100b at 4 and 48 h of life (n=72)

Groups	Serum S100b at 4 h (µg/L)	P-value	Serum S100b at 48h (µg/L)	P-value
Controls(n=20)	1.15 ± 0.16	< 0.001	1.05 ± 0.27	0.001
Cases (n= 52)	3.24 ± 1.83		2.36 ± 1.58	

**Independent sample t-test was used*

measurements were made in µg/L. In the control group mean serum S100b value at 4h was 1.15, which decreased to 1.05 at 48 h. Among the cases group, serum S100b value at 4 h was 3.24, which was decreased to 2.36 at 48 h which was significantly higher than the control group. Comparison of S100b protein in cases and control group has shown statistically significant difference both at 4 h (P-value< 0.001) and 48 h (P-value = 0.001).

Figure 1 shows the comparison between serum S100b levels in the control group, and the different subgroups of cases at 4 h and 48 h respectively. It was found that S100b concentration at 4 h was 1.15 µg/L, 2.06 µg/L, 2.79 µg/L, and 5.12 µg/L in controls, HIE I, HIE II, and HIE IIIrespectively.Similarly, at 48 h it was 1.05 µg/L, 1.35 µg/L, 1.86 µg/Land 4.08 µg/Lin controls, HIE I, HIE II, and HIE III respectively. Thus, the increased levels increased corresponds with the severity of HIE.

Out of 52 cases, 3 neonates from the HIE III group succumbed to death during the treatment and 5 newborns from the control group were lost to follow up. The total number of 64 neonates (15 controls & 49 cases) were followed up until one year of age at 1,3,6,9,12 months of age for neurodevelopment using the DASII score. The below tables show the comparison between children with normal development and those with neurodevelopmental delay concerning mean serum S100b levels at 4 hours (Table 3) and 48 hours (Table 4) of life. The mean S100b levels at 4hours and 48 hours were higher in the developmentally delayed child as compared to developmentally normal children in each of the subgroups.

TABLE 3. Association of Serum S100b at 4h with the long-term outcome (n=64)

Groups	Normal child (M ± SD)	Neuro Handicap (M ± SD)	P-value
Controls(n=15)	1.13 ± 0.15	1.41 ± ---	0.095
HIE I (n=26)	1.64 ± 0.45	4.29 ± 1.04	<0.001
HIE II (n=14)	1.21 ± 0.32	3.55 ± 0.54	0.002
HIE III (n=9)	3.74 ± 1.32	6.50 ± 1.23	0.001

TABLE 4. Association of Serum S100b at 48h with the long-term outcome (n=64)

Groups	Normal child (M ± SD)	Neuro Handicap (M ± SD)	P-value
Controls(n=15)	1.03 ± 0.26	1.41 ± ---	0.187
HIE I (n=26)	1.21 ± 0.32	2.14 ± 0.39	0.001
HIE II (n=14)	1.53 ± 0.46	2.67 ± 0.64	0.001
HIE III (n=9)	3.50 ± 1.32	4.66 ± 1.23	0.030

Table 3 shows the association of serum S100b levels at 4 h with the long-term outcome in the study population. In the control group, the level of serum S100b was almost identical among normal and neuro handicapped children. A significant difference in mean values was observed between normal and neuro handicapped children among HIE I, HIE II, and HIE III categories which are also evidenced by statistically significant p-value.

Table 4 shows the association of serum S100b levels at 48 hrs with the long-term outcomes in the study population. Serum S100b levels were almost identical among normal and neuro handicapped children in the control group. A significant difference was noticed in the mean values of normal and neuro handicapped children among HIE I, HIE II, and HIE III categories which are also evidenced by statistically significant p-value.

FIGURE 1. Comparison of serum S100b at different time intervals in control and HIE groups

TABLE 5. Mean mental and motor DQ (DASII score) at the age of 12 months as per outcome

Variables	Groups	Motor DQ (M ± SD)	Mental DQ (M ± SD)
Normal child (n = 50)	Controls (n=15)	84.5 ± 4.2	86.9 ± 5.6
	HIE I (n=25)	73.5 ± 6.9	79.5 ± 6.3
	HIE II (n=10)	70.0 ± 2.6	74.5 ± 2.7
	HIE III (n=0)	---	---
Neuro Handicap (n = 14)	Controls (n=0)	---	---
	HIE I (n=1)	64.3	70.9
	HIE II (n=4)	54.5 ± 2.3	57.6 ± 5.1
	HIE III (n=9)	37.9 ± 3.0	70.9 ± 2.5

Out of 64 newborns who came for follow-up, 50 newborns had normal neurological outcomes, whereas 14 newborns had developmental delay. Table 5 shows the mean mental and motor DQ scores of the study population at 12 months of age as per the DASII score based on the outcome. Figure 2 shows the area under the curve is 0.80 which is suggestive of high predictability of S100b for the neurological outcome. It was also seen that for a cut-off value of 3.09 µg/L, the specificity was 78.2 % and sensitivity was 80%.

DISCUSSION

Despite the medical and technological advances, HIE continues to be a clinical matter of grave concern owing to its high mortality and morbidity. Recent advances in neuroprotective therapies emphasize the requirement of early markers for diagnosis as well as for predicting the outcome in HIE patients. Several studies have demonstrated an association between the severity of the neuronal injury and the S100b concentration in various body fluids [11-18]. However, there are very few studies that have investigated the S100b levels for asphyxiated newborns, even fewer studies have demonstrated the predictive value of the biomarkers [19-21].

Our study has found that the S100b levels (both at 4 h and 48h) were significantly higher in the case group as compared to the control group. The mean value of S100b levels both at 4h and 48h also increased with the severity of HIE in subgroups. The findings from the study of Thorngren- Jerneck et al and Massora et al [22-24] have shown a similar relationship between S100b levels and the severity of HIE. Some studies have emphasized the early collection of S100b samples owing to the short half-life period of the molecule (30mins) [25]. In our study, we have collected samples both in the early as well as the late neonatal period to observe the trend of the 100b levels in serum. Even though our study has suggested a cut-off value of 3.09µg/L with good sensitivity and specificity as per the ROC curve, it is not possible to accurately predict a normal value warranting the study to be conducted in a larger population. Furthermore, Michetti et al have stated that when high values of S100b are found, the origin other than neuronal tissues should be considered and the low molecular weight of the protein makes it possible to cross the placenta, but the findings regarding this are still controversial.

Nevertheless, considering the devastating consequences of HIE and the availability of neuroprotective therapies, the urgent need for a biomarker to

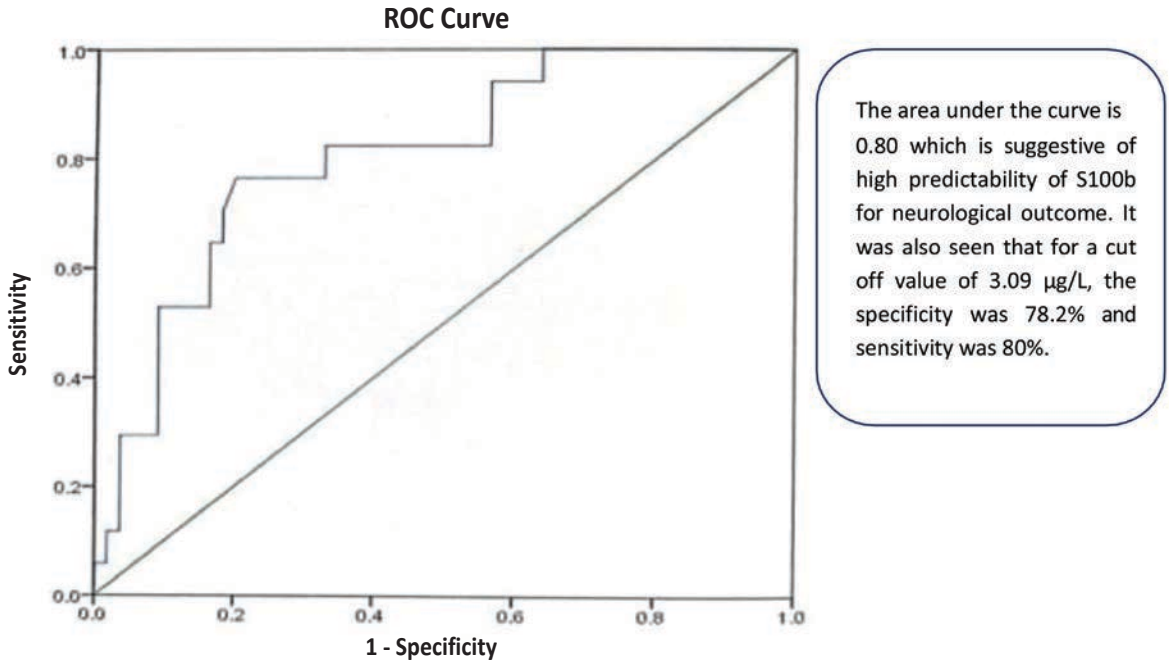

FIGURE 2. ROC curve for serum S100b levels and the neurological outcome of HIE

diagnose and predict the outcome of HIE cannot be undermined. Our study though done in a very limited population clearly states the role that S100b protein may play as an early biomarker.

CONCLUSION

Serum S100b is not only an early biomarker for HIE but may also help in prognostication which is a very essential part of the treatment of newborns as well during the counseling of parents. The result of our study has also demonstrated the role of serum-100bas as a biomarker in diagnosing HIE, assessing the severity, and predicting the neurological outcome. The limitation of this study is the small sample size in which the study was conducted. Hence future studies can be done with much larger sample size and also with umbilical cord blood samples to implement early neuroprotective strategies to prevent neurological sequelae.

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Author's contribution:
The conceptualization and design of the study were done by Dr.Rachita Sarangi and Dr. Subhashree Ray. Material preparation, data collection, and analysis were performed by Dr. O.Y. Pavan Kumar Reddy and Dr. Anup Kumar Rana. The manuscript was written by Dr. O.Y. Pavan Kumar Reddy. Literature search, data analysis and critical review were done by Dr. Rachita Sarangi, Dr. Bibhudatta Dash, and Dr. Subhashree Ray. All authors read and approved the final manuscript.

Consent to participate and publish:
Written informed consent was obtained from the parents.

Conflict of interest:
The authors have no relevant financial or non-financial interests to disclose

Ethics approval:
Approval was obtained from the Institutional ethics committee of the University.

The proceduresused in this study adhere to the tenets of the Declaration of Helsinki.

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