Vol. 4 No. 12 (2023): December 2023 Issue
https://doi.org/10.51168/sjhrafrica.v4i12.913
Original article

PREDICTING MORTALITY OUTCOME IN NEONATES DIAGNOSED WITH HYPOXIC ISCHEMIC ENCEPHALOPATHY: A CROSS-SECTIONAL STUDY.

[1]Saumya Singh, [1]Manisha Kumari*, [2]Bhupendra Narain,
[1]Senior Resident, Department of Pediatrics, P.M.C.H., Patna, Bihar, India
[2]Associate Professor, Department of Pediatrics, P.M.C.H., Patna, Bihar, India

Abstract

Objectives
This study aimed to explore the relationship between varying HIE grades in newborns and levels of reduced glutathione and superoxide dismutase in cerebrospinal fluid, investigating their potential as predictive markers for mortality. The goal was to assess the utility of CSF-based free radical scavengers and antioxidants in predicting mortality in newborns with HIE.

Methods
A 3-year cross-sectional study at Patna Medical Collage and Hospital in Patna, Bihar, India included 86 newborns with hypoxic ischemic encephalopathy. Standard treatments were administered, and CSF analysis for superoxide dismutase and reduced glutathione followed exclusion criteria.

Results
In the study of 140 neonates, 54 were excluded due to consent issues, leaving 86 examined by Sarnat staging. Cerebrospinal fluid superoxide dismutase significantly decreased with HIE severity (81.8, 53.2, 31.6 U/ml, $P < 0.001$). Reduced glutathione exhibited a negative correlation (1354.6, 1041.9, 692.7 ng/ml, $P < 0.001$). Deceased neonates showed significantly lower SOD (61.43 U/ml, $P < 0.001$) and GSH (22.45 U/ml, $P < 0.001$) compared to survivors (1104.32 ng/ml, 584.68 ng/ml, respectively).

Conclusion
The current study reveals that diminished levels of reduced glutathione (GSH) and superoxide dismutase (SOD) in cerebrospinal fluid indicate the intensity of hypoxic ischemic encephalopathy (HIE) and correlate with newborn mortality, highlighting the critical role of oxidative stress. Establishing cut-off values for these antioxidants in CSF may serve as markers for HIE staging and prognosis, guiding the development of targeted neuroprotective therapies for neonates.

Recommendation
The study recommends conducting larger, prospective investigations to address limitations like the small sample size and retrospective design. Furthermore, exploring interventions targeting oxidative stress is advised to enhance outcomes in newborns with HIE.

Keywords: *Birth Asphyxia, Hypoxic Ischemic Encephalopathy, Cerebrospinal Fluid, Superoxide Dismutase*

Submitted: 2023-12-09 *Accepted****: 2023-12-09***

Corresponding author: *Manisha Kumari**

Email:*drmanishahailley@gmail.com*
Senior Resident, Department of Paediatrics, P.M.C.H., Patna, Bihar, India

Introduction
Fetal asphyxia is often linked with neonatal encephalopathy, which is specifically known as hypoxic ischemic encephalopathy (HIE). The occurrence rates of HIE are notably higher in resource-limited countries, contributing to 23% of neonatal deaths globally linked to birth asphyxia [1]. Over a million children with birth asphyxia encounter complications such as brain paralysis, intellectual disability, and cognitive challenges among other [1, 2].

The National Neonatal Perinatal Database Network highlights fetal asphyxia as the predominant contributor of perinatal mortality in intramural live births [3]. Fundamentally, the nerve damage in HIE is a dynamic process, and the ultimate impairment is based on the length and intensity of the initial deterioration, in conjunction with the impacts of ischemia-reperfusion injury as well as cell death [3]. Biochemically, a complex cascade of events unfolds post-HIE damage, presenting a challenge for neonatologists and neurologists in comprehending its pathophysiology and determining the appropriate nature and timing of treatments.

The widely utilized grading system for term babies, developed by Sarnat and Sarnat, forms the basis for recent

evaluation schemes [4]. Numerous attempts have been made to enhance the HIE scoring system, incorporating techniques such as Electroencephalogram, Magnetic Resonance Imaging, MR Spectroscopy, Visual Evoked Response, Ultrasound, Doppler, and Near-Infrared Spectroscopy [5-7]. Enzymes like brain-specific creatine kinase, enolase, and lactate dehydrogenase, are proposed as predictors of outcomes following neurological lesions due to the observed intra- and inter-observer variations in clinical assessments in the absence of reliable markers [8-10].

This study sought to examine the correlation between varying grades of HIE in newborns and the levels of reduced glutathione and superoxide dismutase in cerebrospinal fluid (CSF). Additionally, the study aimed to determine how these CSF-based free radical scavenging enzymes and antioxidants could function as predictive markers for mortality in newborns with HIE.

Materials and Methods

Study design

An epidemiological 3-year cross-sectional study was conducted.

Study setting

The study was conducted at Patna Medical College and Hospital in Patna, Bihar, India, spanning from September 2020 to August 2023.

Inclusion and exclusion criteria

The inclusion criteria comprised infants who had experienced birth asphyxia, subsequently developing hypoxic ischemic encephalopathy. Additionally, the included newborns had a gestational age falling within 37 to 42 weeks, a birth weight ranging from 2-3.5 kilograms, and had survived beyond the initial 12 hours.

Conversely, exclusion criteria were applied to ensure the uniformity of the study population. Newborns with sepsis, major congenital malformations, hemolytic disease, or those experiencing birth trauma were not considered. Furthermore, infants born to mothers with significant health conditions such as severe anaemia, diabetes mellitus, and prior history of medications which can possibly cause drowsiness in newborns, leading to alterations in oxidant or antioxidant levels in blood, were excluded from the study.

Study size

The study included 86 newborns who met specific inclusion criteria.

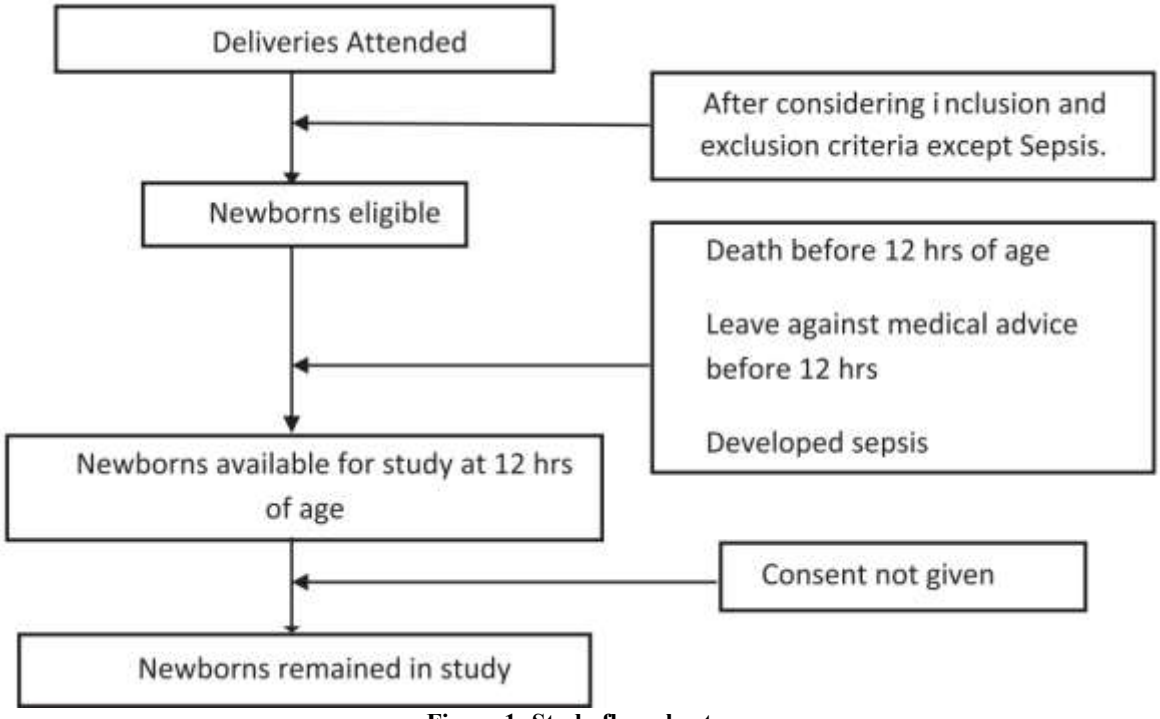

Figure 1: Study flow chart

Data analysis

All participants in the study were administered the standard treatment for hypoxic ischemic encephalopathy (HIE). A comprehensive medical history was compiled, and prior health records were scrutinized. Standard methodologies were applied to document necessary measurements. Gestational age was determined using both menstrual period and the Ballard (II) method. The staging of HIE adhered to the criteria outlined by Sarnat and Sarnat [4].

Within the first 12 to 48 hours of life, cerebrospinal fluid (CSF) was collected for evaluating SOD through Kakkar's modified method and GSH using the approach proposed

by Beutler E et al. [11]. Furthermore, CSF analysis was conducted to exclude the presence of meningitis.

Bias

There was a chance that bias would arise when the study first started, but we avoided it by giving all participants the identical information and hiding the group allocation from the nurses who collected the data.

Statistical Analysis

Statistical analysis used SPSS Statistics 20.0 with p-value lesser than 0.05 considered significant. The data were presented as medians or means, and variances amongst groups were computed via Student's t-test.

Ethical considerations

The study protocol was approved by the Ethics Committee. Prior to the initiation of the observation, informed written consents were acquired, and collaboration was obtained from the parents of all study participants in alignment with the pre-established and pre-tested schedule.

Results/Outcomes

Table 1: Comparison of baseline data between groups

Parameters	HIE-I	HIE-II	HIE-III	P-value
Sex (Male/Female)	10/5	14/5	12/4	0.776
Birth weight (in Kg)	2.52 ± 0.32	2.48 ± 0.25	2.56 ± 0.28	0.773
Gestation (in Weeks)	37.31 ± 0.84	37.47 ± 0.84	37.70 ± 1.02	0.502
Time of collecting CSF (Hrs)	12.92 ± 1.01	13.41 ± 1.27	13.07 ± 1.02	0.425

A total of 140 neonates met the study's inclusion criteria. However, 54 cases were excluded due to the inability to obtain consent. The study proceeded with the examination of 86 neonates, who were then categorized based on the Sarnat and Sarnat staging [4].

Table 2: Values of superoxide dismutase and reduced glutathione with respect to HIE

N	Range	Mean	CV	SE	p-value
SOD, U/ml			**<0.001**		
HIE-I	12	33.98	81.8	12.1	2.56
HIE-II	16	58.12	53.2	34.2	3.98
HIE-III	12	45.43	31.6	41.7	3.41
N	**Range**	**Mean**	**CV**	**SE**	**p-value**
GSH, ng/ml			**<0.001**		
HIE-I	12	851.2	1354.6	19.8	2.56
HIE-II	16	931.5	1041.9	26.6	3.98
HIE-III	12	582.1	692.7	26.9	4.98

A significant and noteworthy difference in cerebrospinal fluid (CSF) levels of superoxide dismutase was observed across the three stages of HIE. The levels of superoxide dismutase demonstrated a substantial decrease with an increase in the severity of HIE, registering values of 81.8, 53.2, and 31.6 SOD U/ml of CSF for the respective stages ($P < 0.001$). Similarly, reduced glutathione in the CSF exhibited a highly negative correlation with the birth asphyxia (p-value < 0.001), with values for the three HIE stages recorded as 1354.6, 1041.9, and 692.7 ng/ml of CSF, respectively (Table 2).

Table 3: Outcomes seen in neonates with respect to physicochemical parameters

Physicochemical Parameters	Neonates survived (n = 74)	Neonates expired (n = 12)	p-value
SOD	61.43	22.45	< 0.001
GSH	1104.32	584.68	< 0.001

Significantly diminished levels of GSH and SOD were observed in newborns who did not survive, in contrast to those who did. The recorded values for SOD were 61.43 U/ml (p-value < 0.001), and for GSH, 22.45 U/ml (p-value < 0.001). The corresponding values for GSH were 1104.32 ng/ml od CSF for survived neonates and 584.68 ng/ml of CSF for the latter cohort (Table 3).

Discussion

In the present study, a significant association was observed between cerebrospinal fluid (CSF) concentrations of glutathione (GSH) and superoxide dismutase (SOD) and neonatal outcomes in cases of birth asphyxia. Notably, neonates who did not survive exhibited prominently lower CSF GSH and SOD concentrations compared to those who survived. These findings suggest a critical link between the extent of injury caused by free radicals and the fate of newborns facing birth asphyxia.

These findings underscores the pivotal role of oxidative stress in determining the prognosis of infants affected by hypoxic ischemic encephalopathy (HIE) due to birth asphyxia. Reduced levels of scavenging enzymes like SOD and antioxidants like GSH in CSF are indicative of heightened oxidative stress, which, in turn, appears to correlate with the severity of HIE and neonatal mortality.

Comparing these findings with prior research, several studies have explored SOD levels in the CSF of neonates with fetal asphyxia and HIE [12-14]. For instance, in a study by Nangia et al., declining SOD levels were observed with increasing severity of birth asphyxia [15]. The recorded SOD values in that study ranged from 30.47 ± 11.8 U/ml in expired neonates to 60.9 ± 24.5 U/ml in normal-survived neonates, with morbid neonates exhibiting intermediate levels [15]. This decline in SOD levels may be attributed to the potential utilization of the protective enzyme in neutralizing or scavenging superoxide radicals, leading to reduced levels in affected newborns.

These findings align with other studies that support the idea of delayed up-regulation of SOD enzyme genes in response to asphyxia [16, 17]. In a separate study where CSF was collected around 72 hours of life, higher SOD activity was reported in infants with HIE compared to controls, suggesting a delay in the up-regulation of SOD genes [18].

While most research on glutathione (GSH) levels in birth asphyxia focuses on blood samples, this study provides valuable insights into CSF GSH levels and their potential relationship with HIE severity. This emphasizes the need for further investigation into the role of oxidative stress and antioxidants like GSH in newborns with HIE. Ultimately, interventions targeting oxidative stress may hold promise for improving outcomes in neonates facing birth asphyxia and HIE.

Conclusion

Oxidative stress is pivotal in determining damage severity in hypoxic ischemic injury. Altered reduced glutathione and superoxide dismutase levels in CSF not only signal hypoxic ischemic encephalopathy (HIE) but also correlate with free radical injury extent. Reduced SOD and GSH levels are linked to severe HIE grades and poor outcomes, making them potential markers. Identifying cut-off values for these antioxidants in CSF holds promise for guiding HIE staging and prognosis, informing the development of targeted neuroprotective therapies for neonates.

Limitations

The current study is constrained by a small sample cohort, retrospective design, potential selection bias, and a focus on CSF without concurrent blood analysis. Larger, prospective studies addressing these limitations are warranted.

Recommendations

The study recommends prioritizing larger, prospective research with diverse populations and concurrent blood analysis to validate findings. Additionally, exploring potential confounding variables, including comorbidities, is recommended to refine understanding of antioxidant levels and hypoxic ischemic encephalopathy severity.

Acknowledgement

To all the participants for their cooperation and patience.

List of Abbreviations

CSF – Cerebrospinal Fluid
HIE - Hypoxic Ischemic Encephalopathy
SOD - Superoxide Dismutase
GSH - Reduced Glutathione
MR- Magnetic Resonance

Source of funding

The study was not funded.

Conflict of interest

No conflict of interest was declared by the author.

References

1. Bryce J, Boschi-Pinto C, Shibuya K, Black RE. WHO estimates of the causes of death in children. Lancet. Mar 26-Apr 1 2005; 365(9465):1147-52.
2. Lawn J, Shibuya K, Stein C. No cry at birth: global estimates of intrapartum stillbirths and intrapartum-related neonatal deaths. Bull World Health Organ. Jun 2005; 83(6):409- 17.
3. National Neonatology Forum: National Neonatal Perinatal Database Report 2002- 03. Published NNPD nodal centre, All India Institute Medical Sciences, New Delhi.
4. Sarnat HB, Sarnat MS: Neonatal encephalopathy following fetal distress: a clinical and electroencephalographic study. Arch Neurol. 1976;33:696–705.
5. Chock VY, Rao A, Van Meurs KP. Optimal neuromonitoring techniques in neonates with hypoxic ischemic encephalopathy. Front Pediatr.

2023 Mar 8;11:1138062. doi: 10.3389/fped.2023.1138062. PMID: 36969281; PMCID: PMC10030520.

6. Elshal, F.I.S., Elshehaby, W.A., Dawoud, M.A.e. et al. Magnetic resonance imaging and spectroscopy in evaluation of hypoxic ischemic encephalopathy in pediatric age group. Egypt J Radiol Nucl Med. 2021, 52, 198.
7. Fleiss B, Wong F, Brownfoot F, Shearer IK, Baud O, Walker DW, et al. Knowledge Gaps and Emerging Research Areas in Intrauterine Growth Restriction-Associated Brain Injury. Frontiers in endocrinology. 2019; 10:188.
8. Fernandez F, Quero J, Verdii A, Ferreiros MC, Daimiel E, Roche MC. LDH isoenzymes in CSF in the diagnosis of neonatal brain damage. ActaNeurolScand 1986; 74: 30-3.
9. DePraeter C, Vanhaesebrouck P, Govaert P, Delanghe J, Leroy J. Creatine kinase isoenzyme BB concentrations in the cerebrospinal fluid of newborns: relationship to short term outcome. Pediatrics 1991; 88: 1204-10.
10. Steinberg R, Gueniau C, Scarna H, Keller A, Worcel M, Pujol JF. Experimental brain ischemia: Neuron-specific enolase level in cerebrospinal fluid as an index of neuronal damage. JfNeurochem 1984; 43: 19-24.
11. Beutler E, Blume KG, Kaplan JC, Lohr GW, Ramot B, Valentine WN. International Committee for Standardization in Haematology: recommended methods for red-cell enzyme analysis. Br J Haematol. 1977;35:331–340.
12. Lemmers PM, Zwanenburg RJ, Benders MJ de Vries LS, Groenendaal F, van Bel F, Toet MC: Cerebral oxygenation and brain activity after perinatal asphyxia: does hypothermia change their prognostic value? Pediatr Res 2013;74:180–185.
13. Si QS, Nakamura Y, Kataoka K: Hypothermic suppression of microglial activation in culture: inhibition of cell proliferation and production of nitric oxide and superoxide. Neuroscience 1997;81:223–229.
14. Gluckman PD, Wyatt JS, Azzopardi D, Ballard R, Edwards AD, Ferriero DM, Polin RA, Robertson CM, Thoresen M, Whitelaw A, Gunn AJ: Selective head cooling with mild systemic hypothermia after neonatal encephalopathy: multicentre randomised trial. Lancet 2005;365:663–670.
15. Nangia S, Saili A, Dutta A K, Batra S, Ray G N. Free Oxygen Radicals –Predictors of Neonatal Outcome Following Perinatal Asphyxia. Indian J Pediatr 1998; 65: 419-27.
16. Cohen G. Enzymatic ~ non enzymaticsources of oxyradicals and regulation of antioxidant defenses. Ann NY AcadSci1991; 563: 8-14.
17. Brawn K, Fridovich I. Superoxide radical and superoxide dismutases : threat and defence. ActaPhysiol Scand. 1980; 492: 9-18.
18. Gulcan H, Ozturk I C, Arslan S. Alterations in Antioxidant Enzyme Activities in Cerebrospinal Fluid Related with Severity of Hypoxic Ischemic Encephalopathy in Newborns. Biol Neonate 2005;88:87-91.
19. Samuelsson M, Vainikka L, Ollinger K. Glutathione in the blood and cerebrospinal fluid: a study in healthy male volunteers. Neuropeptides. 2011 Aug;45(4):287-92.
20. Martini S, Castellini L, Parladori R, Paoletti V, Aceti A, Corvaglia L. Free Radicals and Neonatal Brain Injury: From Underlying Pathophysiology to Antioxidant Treatment Perspectives. Antioxidants (Basel). 2021 Dec 18;10(12):2012.

Vol. 4 No. 12 (2023): December 2023 Issue
https://doi.org/10.51168/sjhrafrica.v4i12.913
Original article

Publisher details

Publishing Journal: Student's Journal of Health Research Africa.
Email: studentsjournal2020@gmail.com or admin@sjhresearchafrica.org

(ISSN: 2709-9997)

Publisher: SJC Publishers Company Limited
Category: Non-Government & Non-profit Organisation
Contact: +256775434261(WhatsApp)
Email: admin@sjpublisher.org
Website: https://sjpublisher.org
Location: Wisdom Centre Annex, P.O. BOX. 701432 Entebbe, Uganda, East Africa.