ORIGINAL RESEARCH

Nigerian Journal of Paediatrics 2024; Volume 51(1): 15-21.
https://dx.doi.org/10.4314/njp.v51i1.03

Cord Blood Hepatic Enzymes as Biochemical Correlates of Hypoxic-Ischaemic Encephalopathy and Immediate Postnatal Outcome in Term Asphyxiated Babies

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Abstract

Background: The untoward effect of perinatal asphyxia on newborns cannot be over-emphasised.

Objective: This study aimed to determine whether hepatic enzymes can serve as biochemical correlates of hypoxic-ischaemic encephalopathy (HIE) and immediate outcomes.

Methods: This cross-sectional study was conducted at the neonatal intensive care unit for 15 months among 70 asphyxiated and 70 healthy neonates. The clinical staging of HIE was based on the Sarnat and Sarnat classification system. A cord blood sample was obtained for the assay of Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), and Lactate dehydrogenase (LDH). The enzymatic assay was performed using the spectrophotometric method.

Results: There was a significant positive correlation between AST ($r = 0.644$, $p < 0.001$), ALT ($r = 0.364$, $p = 0.002$), LDH ($r = 0.377$, $p = 0.001$), and the stages of HIE. AST correlated best with the severity of asphyxia ($r = 0.644$, $p < 0.001$). Of the five mortalities in this series, the cord blood enzyme levels were significantly higher than in those that survived ($p < 0.05$), and all the enzymes demonstrated a positive correlation with mortality, best with ALT ($r = 0.354$, $p = 0.003$).

Conclusion: The worse the degree of perinatal asphyxia in the newborn, the higher the serum hepatic enzymes. Elevation of serum hepatic enzymes may also be associated with the risk of death in such babies. Efforts should be made to prevent severe perinatal asphyxia, and when it becomes inevitable, appropriate and prompt management should be instituted to limit the risk of poor outcomes.

Keywords: Biomarkers, Cord blood, Hypoxic-Ischaemic Encephalopathy, Hepatic enzymes, Perinatal asphyxia.

Introduction

Perinatal asphyxia is now the second leading cause of neonatal deaths after preterm births.[1,2] Globally, 2.3 million children died in the first month of life in 2021, constituting approximately 6400 newborn deaths every day.[3] Multiple organ dysfunction often complicates prolonged perinatal asphyxia, with attendant chronic neurological disabilities among the survivors, especially in developing countries.[4] In an attempt to salvage vital organs following an asphyxia event, there is redistribution of the

cardiac output such that the less vital organs like the liver receive less blood supply. This often results in an early but temporary (within 24 to 72 hours) rise in the liver enzymes, including Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), and Lactate dehydrogenase (LDH) and after that, a steady decline to normal levels after ten days.[5,6] Hypoxic-ischaemic encephalopathy is defined as neonatal encephalopathy following intrapartum hypoxia in the absence of any other abnormality.[7] The defining parameters and stages of HIE are defined by the Sarnat and Sarnat classification system. Thus, considering the untoward effects of hypoxia on the newborn, there is a need to identify babies with a high risk of hypoxic-ischaemic encephalopathy (HIE) and early neonatal death.

The use of the Apgar score alone is limited in predicting postnatal outcomes in asphyxiated neonates, especially long-term sequelae such as cerebral palsy.[8] Hypoxic-ischaemic encephalopathy is an immediate postnatal complication that can be used to describe severe perinatal asphyxia in a term baby and to predict long-term neurological sequelae.[8] Biochemical parameters correlating with the presence of HIE and its stages are desirable. The changes in hepatic enzymes following perinatal asphyxia have been documented by many studies, particularly in developed countries.[9–11] However, only a few studies have correlated the degree of increase in these enzymes with the severity of hypoxic-ischaemic encephalopathy and immediate outcomes in such babies.[12,13] The present study is critical as there is a shortage of published studies in Nigeria on hepatic enzyme activity following hypoxic-ischaemic encephalopathy. The objective of the study was to examine the role of selected hepatic enzymes as biochemical parameters that can correlate with the severity of asphyxia, viz-a-viz the occurrence of hypoxic-ischaemic encephalopathy and its stages, as well as the immediate outcome.

Methods

An analytical, cross-sectional design was used to study 70 term neonates with perinatal asphyxia (with or without HIE) and 70 gestational age- and sex-matched apparently healthy neonates as controls over 15 months. The study was carried out in the inborn section of the Neonatal Intensive Care Unit (NICU) and the Labour Ward of the University of Ilorin Teaching Hospital, Ilorin between August 2020 and October 2021.

Study population

The minimum sample size was determined using the formula to compare two means. A total of 70 asphyxiated and 70 healthy term neonates who fulfilled the inclusion criteria were recruited consecutively.

Inclusion and exclusion criteria for the subjects

The inclusion criteria for the asphyxiated babies included cord blood pH less than 7.2,[14], Apgar score less than seven at the fifth minute of life, and gestational age from 37 to 42 completed weeks. Babies with cord blood pH greater than 7.2, Apgar score greater or equal to seven at the fifth minute of life and those whose mothers/caregivers consented to the study were recruited as controls.

Babies with obvious gross congenital malformations or features suggestive of chromosomal abnormalities or neuromuscular disorders, babies whose mothers use drugs that may affect hepatic enzymes, such as phenytoin, cimetidine, captopril and naproxen, babies whose mothers had prolonged rupture of membranes or features of chorioamnionitis and babies of mothers with active hepatitis were excluded from the study.

Ethical considerations

Ethical approval for the study was obtained from the hospital's Ethics and Research Committee (reference number: NHREC/02/05/2010). After explaining the details and modes of execution of the study to each parent/caregiver, informed consent was obtained from them. The benefits and potential risks of participation were also explained to the parents.

Study procedure

Perinatal asphyxia was diagnosed using cord blood pH and Apgar score (as specified in the inclusion criteria). Hypoxic-ischaemic encephalopathy (HIE) was diagnosed and staged based on abnormal neurological findings that include altered sensorium, altered muscle tone and reflexes, and seizure, as provided in the Sarnat and Sarnat staging system. A cord blood sample was obtained at delivery for the analysis of selected liver enzymes (AST, ALT and LDH). Biochemical assays of these enzymes were performed using an ultraviolet spectrophotometer at the Chemical Pathology Laboratory of the hospital. The hospitalised babies with asphyxia received standard care based on the unit protocols.

Data analysis

The data obtained were entered into a Statistical Package for Social Sciences software version 23 (SPSS Inc., USA) and analysed accordingly. The means and standard deviation (SD) of continuous variables, such as serum levels of AST, LDH and ALT, and the proportion of categorical variables, such as stages of HIE, were determined. The Student's t-test was used to compare the means of continuous variables. Spearman's correlation test was used to show the relationship between the mean values of AST, ALT, and LDH, as well as the stages of HIE and the immediate outcome.

Results

Seventy asphyxiated neonates were recruited with varying degrees of severity. Twenty-two of the neonates had perinatal asphyxia without hypoxic-ischaemic encephalopathy (HIE 0), while the rest had HIE with the distribution as shown in Figure 1. Hypoxic-ischaemic encephalopathy stage II was the most common stage, with a prevalence of 28.6%.

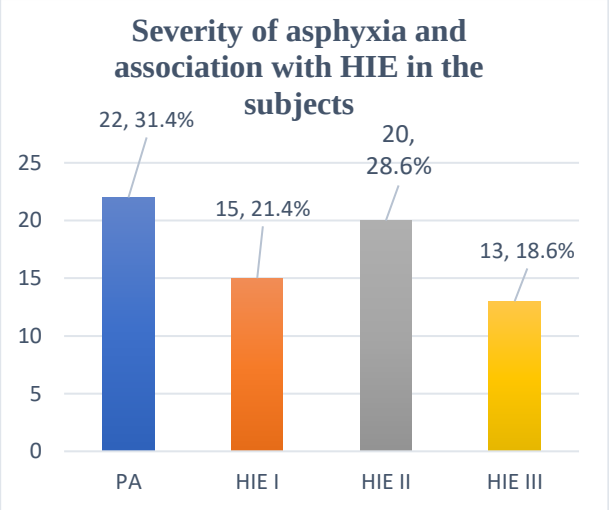

Figure 1. Bar chart showing the severity of perinatal asphyxia and association with HIE in the subjects

Cord blood liver enzyme levels (AST, ALT and LDH) in neonates with asphyxia and healthy controls

The mean serum levels of AST, ALT and LDH were compared between asphyxiated neonates and healthy controls in the cord blood (Table I). The mean levels for all the enzymes were significantly higher in the asphyxiated neonates than in controls ($p < 0.001$).

Table I: Comparison of cord blood liver enzyme levels (AST, ALT and LDH) in neonates with asphyxia (cases) and healthy controls

Variables	**Mean ± SD (U/L)**		**t**	***p*-value**
Cord blood enzymes	Cases	Controls		
AST	85.47 ± 37.83	20.05 ± 9.58	8.46	< 0.001
ALT	25.73 ± 9.56	5.22 ± 1.74	5.75	< 0.001
LDH	483.51 ± 144.16	219.29 ± 77.81	7.21	< 0.001

Correlation of serum levels of AST, ALT and LDH with the stages of HIE

Table II shows a significant positive correlation between AST, ALT and LDH and the stages of HIE (p <0.05). This was strongest for AST (r = 0.644, p <0.001). Besides, AST also correlated significantly with the stages of HIE using only the 48 babies with HIE (HIE I-III) and excluding 22 babies with perinatal asphyxia without HIE (HIE 0), (r = 0.310, p = 0.032). ALT and LDH showed no significant correlation with HIE, p >0.05 in each case.

Correlation of serum levels of AST, ALT and LDH with the immediate postnatal outcome in the asphyxiated neonates

The immediate outcome measured in this study was mortality or survival among the asphyxiated neonates. Table III shows forty-eight (68.6%) neonates had HIE, with HIE stage II accounting for 41.7% of the total HIE cases. Five babies died, all of whom were those with HIE stage III, with a case fatality of 10.4%. Three of the five babies died within 12 to 24 hours of life, while the remaining two died after 24th hours but before the 72nd hour of life.

The mean enzyme levels of AST, ALT and LDH in the cord blood were significantly higher in the babies that died than in those that survived (p <0.05), as shown in Table IV.

Table II: Correlation between stages of HIE and serum levels of liver enzymes in cord blood in neonates with asphyxia

	HIE 0-HIE III	
Enzyme	**r value**	***p*-value**
AST	0.644	<0.001
ALT	0.364	0.002
LDH	0.377	0.001
	HIE I-HIE III	
AST	0.310	0.032
ALT	0.089	0.548
LDH	-0.044	0.766

r: Spearman's correlation coefficient

Table III: Immediate outcome in the asphyxiated neonates

HIE presence	**Outcome n = 70**		
	No of babies, n (%)	***Survived, n (%)***	***Died, n (%)***
No HIE	22 (31.4)	22 (100.0)	0 (0.0)
HIE	48 (68.6)	43 (89.6)	5 (10.4)
Stage I	15 (31.3)	15 (100.0)	0 (0.0)
Stage II	20 (41.7)	20 (100.0)	0 (0.0)
Stage III	13 (27.1)	8 (61.5)	5 (38.5)

Table IV: Correlation and comparison of mean serum AST, ALT and LDH between the survivors and the fatalities

Variables	**Mean ± SD**		**t**	**R**	***p***
Enzymes at birth	Survived	Dead			
AST	79.82 ± 25.54	159.00± 69.44	-2.833	0.325	0.006
ALT	22.86 ± 6.26	63.00 ± 20.57	-3.119	0.354	0.003
LDH	456.74 ± 122.26	831.60±466.95	-2.986	0.341	0.004

t: t-test, r: correlation coefficient.

Discussion

This study used selected hepatic enzymes as biochemical parameters or biomarkers that can correlate with the severity and immediate neonatal outcome of asphyxia. The study revealed that the serum levels of hepatic enzymes (aspartate aminotransferase, alanine aminotransferase, and lactate dehydrogenase) increased significantly with the presence of perinatal asphyxia and also correlated with the stages of hypoxic-ischaemic encephalopathy (HIE) and immediate postnatal outcome.

In this study, more than two-thirds of the asphyxiated babies showed neurological morbidity in the form of HIE, with a higher percentage in Sarnat and Sarnat stage II. This is similar to the findings in a study by another worker,[15] where HIE stage II accounted for the most prevalent stage encountered. In an Indian study,[16] however, it was observed that more babies had HIE stage III. Fatality rates increased with the severity of HIE, as observed in this study and other studies.[17,18] All the deaths in the current study occurred among babies with HIE stage III, compared to the study by other workers, where death was reported in all three stages, though commonest with stage III HIE. [13, 18] Babies with HIE stage III are characteristically with very severe neurological morbidity coupled with other organ dysfunctions.

Cord blood serum levels of alanine aminotransferase, aspartate aminotransferase and lactate dehydrogenase in the present study were significantly higher in the asphyxiated babies than in controls, similar to the report by other workers.[19,13,11] These enzymes are present in the hepatic cytosol, and AST is also found in the mitochondria of the hepatocytes. The increase in their serum levels often noticed in asphyxiated babies may be related to hepatocyte death or increased cell membrane permeability during prolonged hypoxic events, which allows for the efflux of enzymes into the blood.[20] Therefore, investigating these enzymes in the cord blood of babies with suspected asphyxia may help identify those who would eventually have perinatal asphyxia early, and this can allow for prompt and appropriate management, which may improve outcomes in such babies.

In the present study, a significant correlation between the stages of HIE and cord blood hepatic enzyme levels was observed; this was highest with AST. Hypoxic-ischaemic encephalopathy was staged from HIE 0 to HIE III, factoring in all the 70 asphyxiated babies, as used in previous studies.[11, 19, 21] AST still maintained a significant correlation with the stages of HIE even when only the 48 babies with HIE (stages I to III) were analysed. This result suggests that babies with severe perinatal asphyxia, complicated with HIE, develop hepatic hypoxic injury (as indicated by elevated serum enzyme levels). The neurological outcome in such babies may be predicted by the degree of enzyme elevation, especially with AST, as observed in the present study.

Furthermore, the mean cord blood enzyme levels in the asphyxiated babies that died in this study were more than twice that of those who survived, for AST and ALT, while it was almost double for LDH. Therefore, a significant correlation exists between enzyme levels and the chances of death or survival in the immediate postnatal period. This finding is comparable to the report from Ile Ife, Nigeria[13], where the mean AST, ALT and LDH levels obtained at the 12th hour of life were significantly higher in the babies with perinatal asphyxia that died than in the survivors. In a retrospective study conducted on 56 newborns with perinatal asphyxia, elevated serum alanine transaminase (>100U/L) was associated with high mortality in the babies. [9] In another study, the mean serum LDH level was significantly higher in babies with hypoxic-ischaemic encephalopathy who died compared to those who survived, even after both groups had received induced hypothermia treatment. [22] This implies that the degree of multi-organ dysfunction following perinatal asphyxia is also

measurable in the liver and can predict the immediate outcome in affected neonates. A further study that will determine the predictive values and specific cut-off values for the cord blood critical levels of these enzymes in relation to mortality will be desirable. This may prompt early deployment of intensive care that may promote survival in the affected babies. Whereas hepatic injury is reversible in most cases, other organ injuries, including the brain, heart, adrenals and renal, are associated with increased risk of death.

In the present study, five babies out of the 70 asphyxiated babies died, giving a case fatality rate of 7.1 per cent. They all had hypoxic-ischaemic encephalopathy stage III. The case fatality rate was higher than the 5.7 per cent reported in the study conducted at Ile Ife, Nigeria,[13] but lower than some other studies.[22,23] The reasons for these variations may be the differences in the recruitment criteria and the management protocols for the babies at different centres. The current study combined cord blood pH < 7.2 and Apgar score less than seven at the fifth minute of life as the recruitment criteria for asphyxiated babies. In the study done at Ile Ife, an Apgar score of less than seven at the first minute was used as an inclusion criterion for asphyxiated babies, unlike the same score at the fifth minute in the present study. This may bring about some disparities in the reported outcomes, as babies with persistently low Apgar scores up to the fifth minute are likely to have a worse outcome, hence more deaths.

Conclusion

It is concluded that serum levels of liver enzymes can be used as biomarkers of the severity of perinatal asphyxia and can be employed in predicting the presence of HIE, as well as the immediate postnatal outcome in asphyxiated babies. It is recommended that serum ALT, LDH and especially AST levels should be estimated in babies with asphyxia who are delivered in the hospital and those referred with a history suggestive of asphyxia to determine the severity and thus initiate early intervention to prevent further neurological damage.

This study had the strengths of being a prospective study, with the inclusion of gestational age and a sex-matched healthy control group. Also, it utilised both the Apgar score and cord blood pH as the primary inclusion criteria for asphyxiated babies. Unfortunately, long-term follow-up of the babies with HIE who survived was not included in the study, so they could not be monitored for long-term sequelae.

Acknowledgement: The authors appreciate the assistance of the resident doctors at the Department of Paediatrics, University of Ilorin Teaching Hospital, with data collection. Mr. Ogunwale Kolawole of the Chemical Pathology Laboratory is also acknowledged for his assistance with laboratory procedures.

Authors' contributions: ASO, OAI, AOO, AOV, BSA and BAO conceptualised the study. ASO, OAI, AOO and AOV collected and analysed the data while ASO interpreted the data. ASO drafted the manuscript while OAI, AOO, AOV and BAO revised the draft. All the authors approved the final version of the manuscript.

Conflict of interest: None declared.

Financial support: The authors received no funding for the research and publication of this article.

Accepted for publication: 30th March 2024

References

1. World Health Organization (2019). Newborns: reducing mortality. WHO Fact Sheets. http://www.who.int/news-room/fact-sheets/detail/newborns-reducing-mortality. Accessed 20 August 2023.
2. Liu L, Johnson HL, Cousens S, Perin J, Scott S, Lawn JE *et al.* Global, regional, and national causes of child mortality: An updated systematic analysis for 2010 with time trends since 2000. *The Lancet* 2012; 379: 2151–2161.
3. UNICEF. UNICEF Data: Monitoring the situation of children and women. UNICEF, New York. 2018.
4. Padayachee N, Ballot DE. Outcomes of neonates with perinatal asphyxia at a tertiary academic hospital in Johannesburg, South Africa. *South Afr J Child Health* 2013;7:89–94.

5. Ciobanu AO, Gherasim L. Ischemic Hepatitis – Intercorrelated Pathology. *Maedica (Bucur)* 2018;13:5.
6. Giannini EG, Testa R, Savarino V. Liver enzyme alteration: a guide for clinicians. *CMAJ* 2005;172: 367–379.
7. AAP Committee on Fetus and Newborn, ACOG Committee on Obstetric Practice. Neonatal Complications and Management of High-Risk Infants: Guidelines for Perinatal Care. 7th Edition. American Academy of Paediatrics; 2012. p.321-382.
8. Levene MI, Grindulis H, Sands C, Moore JR. Comparison of Two Methods of Predicting Outcome in Perinatal Asphyxia. *The Lancet* 1986; 327: 67–69.
9. Tarcan A, Tiker F, Güvenir H, Gürakan B. Hepatic involvement in perinatal asphyxia. *J Maternal-Fetal Neonat Med* 2007;20:407–410.
10. El-Kabbany ZA, Hamza R, Toaima N. Early Hepatic Dysfunction in Asphyxiated Full-Term Newborns. *SM J Gasrtoenterol Hepatol* 2017;2:1–5.
11. Choudhary M, Sharma D, Dabi D, Lamba M, Pandita A, Shastri S. Hepatic dysfunction in asphyxiated neonates: a prospective case-controlled study. *Clin Med Insights Pediatr* 2015;9:1–6.
12. Patra C, Sarkar S, Dasgupta M. Study of hepatic enzyme activity as a predictor of perinatal asphyxia and its severity and outcome. *Indian J Health Sci* 2016;9:297.
13. Ogundele T, Oseni SBA, Owa JA, Ogundele O. Lactate Dehydrogenase, Aspartate Aminotransferase, and Alanine Aminotransferase Cord Serum Levels as Early Markers of Hypoxic–Ischemic Encephalopathy in Babies with Severe Perinatal Asphyxia. *J Pediatr Neurol* 2019;17:105–110.
14. Leuthner SR. Low Apgar scores and the definition of birth asphyxia. *Pediatr Clin North Am* 2004;51:737–745.
15. Finer NN, Robertson CM, Richards RT, Pinnell LE, Peters KL. Hypoxic-ischemic encephalopathy in term neonates: Perinatal factors and outcome. *J Pediatr* 1981;98:112–117.
16. Singh N, Gupta AK, Arya AK. A Study on the Correlation of Umbilical Cord Arterial Blood pH with Perinatal Asphyxia and Early Neonatal Outcome. *Asian J Clin Pediatr Neonatol* 2020;8:30–35.
17. Babu BVA, Devi SS, Kumar BK. Birth asphyxia – Incidence and immediate outcome in relation to risk factors and complications". *Int J Res Health Sci* 2014;2:1064–1071.
18. Ezenwa BN, Olorunfemi G, Fajolu I, Adeniyi T, Oleolo-Ayodeji K, Kene-Udemezue B *et al.* Trends and predictors of in-hospital mortality among babies with hypoxic ischaemic encephalopathy at a tertiary hospital in Nigeria: A retrospective cohort study. *PLoS One* 2021;16:e0250633.
19. Karlsson M, Blennow M, Nemeth A, Winbladh B. Dynamics of hepatic enzyme activity following birth asphyxia. *Acta Paediatr* 2006;95:1405–1411.
20. Chhavi N, Zutshi K, Singh NK, Awasthi A, Goel A. Serum Liver Enzyme Pattern in Birth Asphyxia-Associated Liver Injury. *Pediatr Gastroenterol Hepatol Nutr* 2014;17:162.
21. Elsadek AE, FathyBarseem N, Suliman HA, Elshorbagy HH, Kamal NM, Talaat IM *et al.* Hepatic Injury in Neonates with Perinatal Asphyxia. *Glob Pediatr Health* 2021;8:2333794X20987781.
22. Thoresen M, Liu X, Jary S, Brown E, Sabir H, Stone J *et al.* Lactate dehydrogenase in hypothermia-treated newborn infants with hypoxic-ischaemic encephalopathy. *Acta Paediatr, Int J Paediatr* 2012;101:1038–1044.
23. Tarcan A, Tiker F, Güvenir H, Gürakan B. Hepatic involvement in perinatal asphyxia. *J Matern Fetal Neonat Med* 2007;20:407–410.