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Use of serum levels of selected enzymes as a supportive tool in assessing severity of birth asphyxia in low resource setting

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Abstract*:* *Introduction:* Severe birth asphyxia is one of the reasons why babies are admitted into the newborn unit and contributing significantly to neonatal morbidity and mortality. Hypoxic injury, when severe, leads to leakage of intracellular enzymes into the circulation. The level of these enzymes reflects the severity of the damage; this can identify babies with a severe injury, especially the out borns whose deliveries were not supervised. This study aimed to relate the serum levels of three enzymes at the age of twelve hours to the severity of birth asphyxia using the Apgar score and neurological state of the babies.

Methods: A prospective comparative cross-sectional study. Term babies with Apgar score < 7 at 1-minute of life were recruited, scores of 0-3 were taken as severe birth asphyxia. Serum levels of lactate dehydrogenase, aspartate aminotransferase, and alanine aminotransferase, were determined at the age of 12 hours using an ultraviolet spectrophotometer. Levels of the enzymes were related to the severity of birth asphyxia. SPSS for Windows, version 18 was used to analyse the data

Results: Seventy babies with birth asphyxia and 70 controls were studied. Fifteen (41.7%) of the 36 babies with severe birth asphyxia had hypoxic-ischemic encephalopathy, four (5.7%) of which died. The mean values of each of the enzymes were higher in babies with hypoxic-ischaemic encephalopathy than in those without ($p = 0.001$), and in babies that died than babies that survived ($p = 0.001$).

Conclusion: Estimation of these enzymes clearly defines the severity of hypoxic injury in babies with birth asphyxia. The estimation of these enzymes will be a useful tool in identifying babies with birth asphyxia especially in outborns whose deliveries were not supervised.

Keywords: Birth asphyxia, lactate dehydrogenase, aspartate aminotransferase and alanine aminotransferase.

Introduction

Birth asphyxia (BA) is an insult to the fetus or newborn due to a lack of oxygen or perfusion to various organs. It is associated with lactic acidosis, hypoxia, and hypercapnia.[1] The methods of evaluating the severity and predicting outcomes of BA are based on the conditions of the baby at birth using Apgar scores and blood gas analysis. [2,3] There is no facility for blood gas analysis in most hospitals in low resource settings. Therefore, the Apgar score remains the only tool used to assess babies at birth. Thus, the assessment of the severity of the insult from BA is a challenge, especially in outborns whose Apgar scores were not done at birth. Hence, other methods, such as the use of levels of biochemical markers that correlate with the severity of the hypoxic-ischemic insult, are desirable. When there is a prolonged hypoxic-ischemic insult, it may damage multiple organs leading to leakages of intracellular enzymes into the circulation.[4-12] There is a dearth of such study in Nigeria and other developing countries where severe BA is more common. The present study aims to determine the serum levels of lactate dehydrogenase (LDH), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) at the age of 12 hours and evaluate their usefulness in assessing the severity of hypoxic-ischemic injury in babies with BA. LDH, AST and ALT are produced from multiple organs including the liver, heart, lungs, brain, kidneys and skeletal muscles.

Material and method

Ethical Consideration: An ethical approval with an ethical clearance certificate (with a protocol number

ERC/2013/07/05) was obtained from the Ethics and Research Committee. Informed consent was obtained from the mother of each baby.

Study setting: The study was carried out at the labour and neonatal wards of a tertiary hospital in South-West, Nigeria. The hospital is a major referral health facility providing neonatal and obstetrics care.

Study design: This was a prospective, comparative, cross-sectional study. We determined the sample size using the formula for calculating the sample size for comparing a continuous variable between subjects and controls, and the minimum sample size was 32 babies per group.[13] The controls were age- and sex-matched babies without BA. The sample size was doubled to 64 babies to improve the validity of the results.[14,15] This number was rounded up to 70. Thus, 70 babies with BA and 70 controls were recruited into the study.

Recruitment of subjects: The Apgar score of consecutive term babies delivered at the labour ward was determined by the investigator. In this study, babies with Apgar scores ≤ 6 at one minute were recruited as babies with BA, while an equal number of babies, sex- and age-matched with Apgar scores of ≥ 8 were recruited as controls. The controls were otherwise apparently healthy term babies. Severe BA was defined as an Apgar score of ≤ 3 while moderate BA was defined as 4- 6 at one minute of life.[16,17]

Babies with an obvious congenital malformation or neuromuscular abnormalities, those whose mothers were given sedative drugs like diazepam or magnesium sulphate within four hours prior to delivery and those whose mothers had prolonged rupture of membranes or features of chorioamnionitis were excluded from the study.

Detailed clinical and neurological evaluations of the babies were carried out at birth. Babies with BA were admitted and monitored in the special care baby unit of the hospital. The gestational age (GA) was determined by the mother's last menstrual period or early ultrasound done. The birth weight in kilogram was taken with Seca digital weighing scale, model 724 measuring to the nearest 0.1kilogram. Features of hypoxic-ischemic encephalopathy (HIE) were carefully documented and graded based on Levene's classification of HIE using the level of consciousness, tone, the presence of seizure, sucking, and the respiration pattern.[18]

Data analysis: The data were analysed using Statistical Package for Social Sciences (SPSS) for Windows version 18. Means and standard deviations (SD) were determined for continuous variables such as weight and serum levels of the enzymes, while proportions and percentages were determined for discrete variables such as sex, the severity of BA, grades of HIE, and the outcome of hospitalization. Means were compared using Student's t-test. Proportions and ratios were compared using Pearson's Chi-squared ($\chi 2$) test. A statistically significant level was set at a p-value < 0.05 in two-tailed tests.

Results

Study population

A total of 140 babies were recruited into the study. They comprised 70 babies with BA (36 with severe BA and 34 with moderate BA) and 70 controls. Among the babies with BA, 38 (54.3%) were males giving a male: female ratio of 1.2: 1. and the same for the control

Gestational age: The gestational ages were between 38 and 40 weeks. The means (SD) of gestation ages of the neonates with BA and controls were 39.2 (1.0) weeks and 39.3 (1.0) weeks. The difference was not statistically significant ($t = 0.507$, $p = 0.613$).

Birth weight: The birth weights ranged between 2.5 and 4.0kg. The means (SD) of birth weights of the newborns with BA was 3.2 (0.36) kg as compared with 3.2 (0.33) kg for the controls. The difference between the means (SD) of birth weights of the two groups was not statistically significant ($t = 0.00$; $p = 1.00$).

The severity of birth asphyxia

Data on the severity of birth asphyxia are shown in Table 1. Among the 70 babies with BA, 36 (51.4%) had a severe BA, while 34 (48.6%) had a moderate BA. Fifteen (41.7%) of the 36 babies with severe BA had HIE. Two (13.3%) of the 15 babies with HIE had mild, five (33.3%) had moderate, and eight (53.4%) had severe. Four (26.7%) of the 15 babies with HIE died, and the four deaths occurred among the eight babies with severe HIE. The mortality rate in babies with severe HIE was, therefore, 50.0%. There was no death in any of the other groups.

Table 1: Distribution of Grades of Severity of BA and HIE among the 70 Babies with BA

Birth asphyxia	HIE	Number (%)	Death (%)
Moderate (n = 34)	None	0 (0.0)	0 (0.0)
Severe (n = 36)	Mild	2 (2.9)	0 (0.0)
	Moderate	5 (7.1)	0 (0.0)
	Severe	8 (11.4)	4 (50.0)
Total with HIE	-	15 (21.4)	4 (26.7%)
Severe BA without HIE	No	21 (30.0)	0 (0.0)
Moderate (n = 34)	No	34 (48.6)	0 (0.0)
Grand total		70 (100.0)	4 (5.7%)

The range of the enzyme Levels

Table 2 shows the ranges of the enzyme levels and the comparisons of the means of serum levels of LDH, AST, and ALT between patients and controls. The means (SD) of serum LDH, AST, and ALT in babies with BA were statistically significantly higher than those for controls ($p = 0.000$).

Table 2: Comparison of the Ranges and Means of Serum Levels of LDH, AST and ALT at the Age of 12 Hours between Babies with BA and the Controls.

Enzymes	Ranges Patients (n=70)	Control (n=70)	Mean (SD) U/L Patients (n = 70)	Controls (n = 70)	t	p value
LDH	389.1-1490.0	112.0 - 625.0	794.4 (280.5)	258.0 (95.8)	15.15	0.000
AST	20.6 - 319.0	18.0 - 57.3	70.5 (47.8)	32.9 (10.9)	6.41	0.000
ALT	8.3- 106.0	5.6- 47.0	38.3 (19.7)	17.2 (9.0)	6.82	0.000

Table 2: Comparison of the Ranges and Means of Serum Levels of LDH, AST and ALT at the Age of 12 Hours between Babies with BA and the Controls.

	Moderate BA (n = 34)	Severe BA group (n = 36)	Severe BA without HIE (n = 21)	Severe BA with HIE (n = 15)
LDH	602.7 (153.7) P < 0.001	975.3 (251.8)	790.1 (124.8) P < 0.001	1234.7 (112.9)
AST	45.0 (18.8) P < 0.001	94.6 (54.3)	67.5 (21.3) P < 0.001	132.5 (63.8)
ALT	29.2 (13.2) P < 0.001	46.9 (21.1)	33.8 (13.6) P < 0.001	65.2 (15.1)

The mean value of the enzymes

Table 3 shows the comparisons of the means of serum levels of LDH, AST, ALT in neonates with moderate and severe BA, then in neonates with HIE, and without HIE among babies with severe BA. The means of serum levels of LDH, AST, and ALT among babies with severe BA were statistically significantly higher than babies with moderate BA and also, in babies with HIE than in babies without HIE.

The best LDH, AST and ALT cut off values, calculated in the ROC curve, for prediction of HIE is given in Table 4 together with the predictive values. LDH, AST and ALT offered high sensitivity, specificity and predictive value for the prediction of HIE. The most suitable marker for the prediction of HIE was LDH.

Table 5 shows the comparisons of the means of LDH, AST, and ALT levels between babies that survived and babies that died among babies with BA. The means of serum levels of LDH, AST, and ALT in babies that died were significantly higher than those that survived. Those that died had 1.5 times LDH levels, 3 and 2.25 times AST, and ALT levels, respectively, than those who survived.

**Parents of one of the babies with severe BA discharged against medical advice before the age of 24 hours, so the final outcome was not known.

The median values in relation to the severity of BA

Figure 1 shows the Box-and-Whisker plots of LDH in blood samples of 15 babies with HIE and the 55 babies without HIE among the 70 babies with BA. The Box-and-Whisker plot for LDH shows clearly that at 12 hours, the median value and two standard deviations for babies without HIE were below those of babies with HIE.

Figure 2 shows the Box-and-Whisker plots of AST for 15 babies with HIE and the 55 babies without HIE among the 70 babies with BA. The Box-and-Whisker plots for AST show more clearly that at 12 hours, the median values and two standard deviations for babies without HIE were below those of babies with HIE.

Figure 3 shows the Box-and-Whisker plots of ALT for 15 babies with HIE and the 55 babies without HIE among the 70 babies with BA. The Box-and-Whisker plot of ALT shows more clearly at 12 hours that median values and two standard deviations for babies without HIE were below those of babies with HIE.

Table 4: Best cut off values for enzymes obtained from a receiver operating characteristic curve (ROC) presented together with area under the curve (AUC)

Severe BA included (No = 36)	LDH	AST	ALT
AUC	1.000	0.932	0.975
Cut off	1006.8U/L	88.8U/L	49.5U/L
Sensitivity	100%	86.7%	93.3%
Specificity	100%	90.5%	95.2%
PV^+	100%	92.9%	100%
PV^-	100%	90.9%	95.5%

Table 5: The Comparison of the Means of LDH, AST and ALT Levels between Babies that Survived and Babies that Died among Babies with BA.

Enzymes	Mean (SD) U/L Survivors (n = 65)	Died (n = 4)	t	pvalue
LDH	768.0 (266.0)	1228.5 (96.1)	3.428	< 0.001
AST	62.8 (30.4)	198.0 (97.8)	7.919	0.000
ALT	35.7 (16.6)	81.3 (16.9)	5.320	0.000

**Parents of one of the babies with severe BA discharged against medical advice before the age of 24 hours

Fig 1: Showing Box-and-Whisker plot of LDH for blood samples at 12 hours for 15 babies with HIE and 55 babies without HIE among the 70 babies with PA

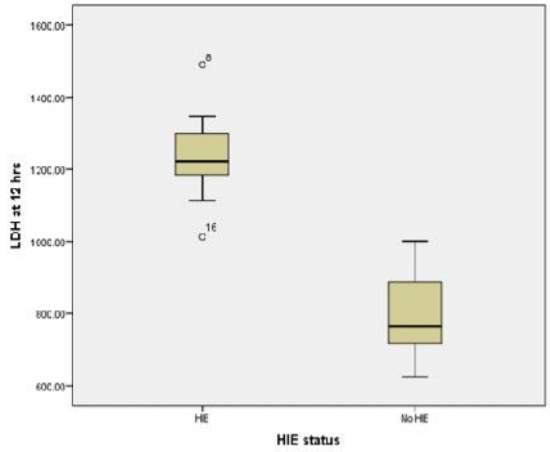


Fig 2: Showing Box-and-Whisker plot of AST for blood samples at 12 hours for 15 babies with HIE and 55 babies without HIE among the 70 babies with PA

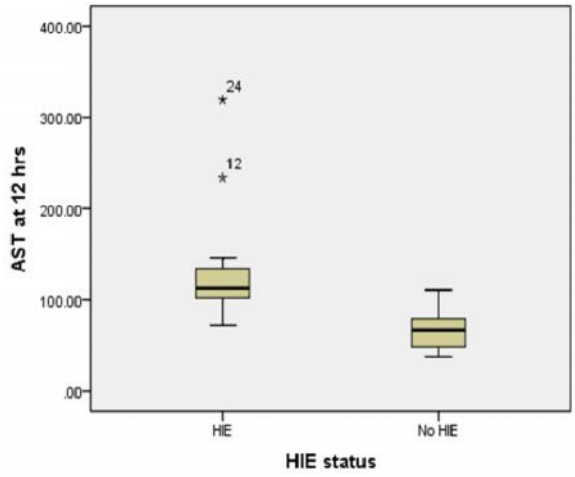
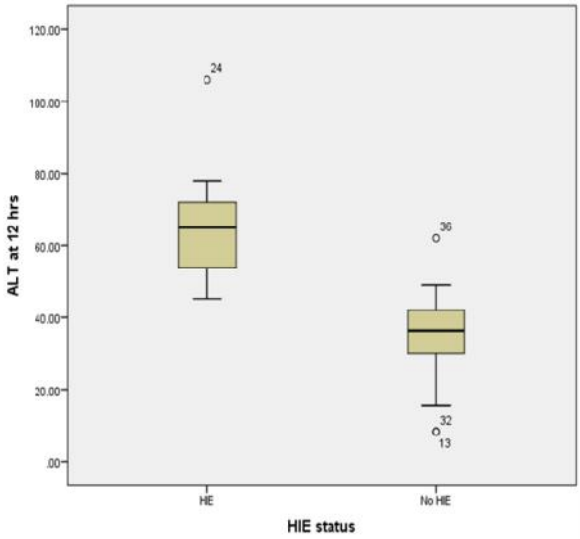


Fig 3: Showing Box-and-Whisker plot of ALT for blood samples at 12 hours for 15 babies with HIE and 55 babies without HIE among the 70 babies with PA



Discussion

The present study has provided data on serum values of LDH, AST, and ALT at 12 hours of life in apparently healthy newborns and those with varying severities of BA. It also assessed the use of serum levels of LDH, AST, and ALT at 12 hours as a tool to evaluate the severity of hypoxic-ischaemic injury in babies with BA.
It is a common experience that many babies are born outside the orthodox hospitals and are not assessed using Apgar scores since the attendants of such births are not skilled in such an assessment of the newborn babies. Such omissions often occur in the private hospital, mission houses, and nursing home settings where the attending midwives are not closely supervised or are even auxiliary nurses. Such babies present in the hospital with various complaints, ranging from poor cry to abnormal body movements. The severity of the insult, most time, could not be ascertained. The management of such babies depends on the severity from close observation to neuroprotective measures.

The three enzymes assayed in the present study are usually released from several organs following hypoxic injury.[5-7] There was no facility to assay for organ-specific LDH iso-enzyme in this centre, so total serum LDH level was assayed.
In the present study, at 12 hours of life, the means of these enzymes, LDH, AST, and ALT, were statistically significantly higher in neonates with BA than those without BA. This finding corroborated the results from previous studies.[19-23] The elevated serum levels of the enzymes in babies with BA supported the suggestion that there are leakages of the intracellular enzymes, including LDH, AST and ALT, into the circulation in response to hypoxic-ischemic injury.[5-7] In addition, increasing values of means of the enzymes with the severity of BA suggested a dose-response relationship between the serum levels of the enzymes and the severity of BA. Therefore, the serum levels of the enzyme may be helpful in the retrospective diagnosis and assessment of the severity of BA.

At the age of 12 hours in the present study, the means of serum values of LDH in the babies with severe BA who had developed HIE was statistically significantly higher than that in babies with severe BA without HIE. The results in the present study were comparable to results of previous studies, despite the differences in the ages at which samples were taken.[18,19,24,25]Karuntilaka et al. took samples within the first six hours of life, while Karlsson et al. and Choudhary et al. sampled their babies within 72 hours of life. [5,24,25] Thus, while the hypoxic injury occurred around the time of delivery, assaying for these enzymes at 12 hours can still yield valuable information for accurate assessment of the severity of the hypoxic injury and appropriate management or decision-making.

The high serum levels of these enzymes from different

studies strongly suggest that babies who developed HIE had suffered a more severe hypoxic-ischemic injury involving multiple organs, resulting in the release of a higher amount of the enzymes into the circulation.[26,27] The means of serum levels of AST and ALT levels at 12 hours in babies with HIE were significantly higher than those of babies without HIE ($p = 0.000$). Islam and many other researchers had shown a similar relationship between the levels of the enzymes, the severity of BA and HIE.[23,26,28] Thus, the levels of the enzymes were able to separate babies with severe BA into two groups of those that developed HIE and those without HIE. Serum levels of the enzymes at 12 hours of life in babies who developed HIE were higher than those without HIE in this study. The cutoff values of LDH, AST and ALT for predicting HIE in this study were similar to what was reported by Karlsson et al.[19] In the present study, the sensitivity, specificity, positive predictive values and negative predictive values of the enzymes for diagnosing HIE were very high. For LDH, the sensitivity and specificity were 100% respectively, strongly supporting its usefulness for this purpose.

The means of serum levels of LDH, AST, and ALT in the four babies that died were significantly higher when compared with those that had BA but survived. This was comparable with the report by Thoresen et al., who compared the plasma levels of LDH in those that died with those that had HIE but survived.[28] Tarcan et al. also recorded the highest activity of ALT among babies that had HIE and died[20] Godambe et al. reported that more babies with raised ALT died than those who survived.[29] The babies who died had suffered a more severe hypoxic -ischemic injury, which caused more severe organ damage, resulting in more enzymes in these babies than in the other babies with severe BA but survived.

The strength of this study is in the usage of the standard enzyme assay method in the diagnosis and assessment of the severity of BA, which may be very useful in a low resource setting like ours where blood gas analysis is not available. The limitation of the study is in the usage of the Apgar score as a lone measure in defining BA. This was, however, minimized by the principal investigator, who did all the Apgar scoring.

Conclusion

The present study clearly showed that serum levels of these enzymes at the age of 12 hours could give a clue to the presence and severity of hypoxic injury in babies with severe birth asphyxia and this can serve as an adjunct tool to the assessment of the severity of hypoxic-ischaemic injury in babies with birth asphyxia in a low resource setting.

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