Lactate Dehydrogenase, Aspartate Aminotransferase, and Alanine Aminotransferase Cord Serum Levels as Early Markers of Hypoxic–Ischemic Encephalopathy in Babies with Severe Perinatal Asphyxia

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Abstract

Perinatal asphyxia is a major cause of morbidity and mortality among newborn babies. Severe perinatal asphyxia can be associated with multiple organ dysfunctions resulting in the release of a variety of intracellular enzymes. A major concern is how to identify newborns in need of prompt and aggressive management to minimize the risk of early severe neurological sequelae such as hypoxic–ischemic encephalopathy. The present study was performed to determine the relationship between cord serum levels of lactate dehydrogenase, aspartate aminotransferase, alanine aminotransferase, and severity of perinatal asphyxia among Nigerian newborn babies. This was a prospective, comparative case–control study at the Obafemi Awolowo University Teaching Hospital, Ile-Ife. Cord blood was collected at delivery for serum levels of lactate dehydrogenase, aspartate aminotransferase, and alanine aminotransferase. Each baby was evaluated for the severity of perinatal asphyxia at 1 minute of life using Apgar scores. Apgar score less than 7 at 1 minute was regarded as perinatal asphyxia. The Apgar scores were related to cord serum levels of the enzymes. The data were analyzed using Statistical Package for the Social Sciences for Windows, version 17.0. One hundred and forty babies, comprising 70 babies with and 70 babies without perinatal asphyxia were studied. Thirty-six (51.4%) of the neonates had severe perinatal asphyxia with Apgar score of 3 and below; 15 (41.7%) of the 36 had hypoxic–ischemic encephalopathy. The mean of values of each of the three enzymes was statistically significantly higher in babies with perinatal asphyxia compared with controls ($p < 0.001$ for each enzyme) and in babies with hypoxic–ischemic encephalopathy than in babies with severe perinatal asphyxia but without hypoxic–ischemic encephalopathy ($p < 0.001$). A very high proportion of babies with severe perinatal asphyxia developed hypoxic–ischemic encephalopathy. Based on the cord serum enzyme levels, almost all the babies who had hypoxic–ischemic encephalopathy would have been identified at delivery. Routine estimation of the cord serum levels of these enzymes among babies with severe perinatal may be used to identify babies who may develop acute serious neurological complications for anticipatory management.

Keywords

► cord serum enzyme levels
► Apgar scores
► perinatal asphyxia
► hypoxic–ischemic encephalopathy

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ISSN 1304-2580.
Introduction

Perinatal asphyxia (PA) occurs when a newborn fails to initiate and/or sustain spontaneous breathing in the first minute of life, resulting in low oxygen supply (hypoxia) and/or poor perfusion (ischemia) of various organs with consequences such as hypoxemia, hypercapnia, and acidosis. PA is a leading cause of neonatal mortality and chronic neurological disability among the survivors, especially in developing countries. Globally, approximately 23% of neonatal deaths are due to PA, while in Nigeria, approximately 26% of deaths are attributable to PA.

The World Health Organization definition of birth asphyxia in International Classification of Diseases (ICD-10) is based on 1-minute Apgar score. The Apgar score of 0 to 3 is considered severe, while a score of 4 to 7 is taken as mild-to-moderate PA. The American College of Obstetrics and Gynecology and American Academy of Pediatrics label a neonate as having severe PA if all the following conditions are satisfied:

1. Umbilical cord arterial pH less than 7;
2. Apgar score of less than 3 for longer than 5 minutes;
3. Neonatal neurological manifestations (e.g., seizures, coma, or hypotonia);
4. Multisystem organ dysfunction (MOD), involving cardiovascular, gastrointestinal, hematological, pulmonary, and renal systems.

During a hypoxic-ischemic insult, the fetal circulatory system will divert cardiac output to the brain, heart, and adrenals at the expense of other organs such as the kidney, gastrointestinal tract, skin, liver, muscles, and lungs. Cellular damages to multiple organs are the consequences of such diversion, with resultant leakages of intracellular enzymes such as lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, creatine kinase, and many other enzymes across the abnormally permeable cell membranes into the circulation. The enzyme levels in the circulation provide important parameters of cell injury that can then be used to identify babies with MOD, especially hypoxic-ischemic encephalopathy (HIE). The organs that are often affected are the lungs in 86%, liver in 85%, kidneys in 70%, and heart in 62% of cases.

LDH catalyzes two-way conversion between pyruvate and lactate with concomitant two-way conversion between NADH and NAD+. LDH converts pyruvate, the final product of glycolysis, to lactate when oxygen is absent or in short supply, and performs the reverse reaction during the Cori cycle in the liver. In serum, LDH has five different isotypes (numbered 1–5) that differ slightly in structures. LDH-1 is highest in the heart, red blood cell, and brain; LDH-2 is concentrated in the white blood cells; LDH-3 is highest in the lungs; LDH-4 is concentrated in the kidney, while LDH-5 is abundant in the liver and skeletal muscles. Elevated levels of serum LDH have been observed in many medical conditions such as megaloblastic anemia, muscular disorders, nephritic syndrome, liver cirrhosis, and leukemia. Serum LDH levels may be elevated following myocardial or pulmonary infarction, or hypoxic hepatitis, which may be seen in babies with PA. Total serum LDH, which includes all the isoenzymes, was assayed in this study. The normal range for total LDH from birth through the first year of life is 170 to 580 U/L.

AST transfers an amino group from glutamate to oxaloacetate thereby forming aspartate. The enzyme is found in hepatic, myocadial, and kidney tissues. Hypoxic-ischemic injury and other diseases of these organs, including hepatobiliary conditions, ischemic heart diseases, viral hepatitis, and hypoxic hepatitis, lead to an increase in plasma levels of AST. Two isoenzymes of AST have been reported, one in the cytoplasm and the other in the mitochondria. It is only the cytoplasmic AST that is normally found in plasma. The normal range in the first 5 days of life is 35 to 140 U/L.

ALT catalyzes the reaction where the amino acid, alanine loses its amino group by transamination to form pyruvate. The liver is the major source of ALT, but it is also produced in very small amounts in other tissues. Therefore, ALT is considered a more specific liver enzyme. An increased plasma level of ALT is seen in different forms of hepatic diseases including hypoxic hepatitis seen in PA. The normal range in the first 5 days of life is 6 to 50 U/L.

Apgar score is useful for the recognition of a baby with PA, but the Apgar score has limitations. There is need for biomarkers that can define the presence and severity of asphyxia in babies with severe PA, especially when the labor is not supervised by skilled personnel. Consequently, several studies have been undertaken to determine the relationship between intracellular enzymes and PA. There is dearth of data in Nigeria, one of the major countries where PA is a major problem. The present study, therefore, aimed at determining the relationship of these enzymes with presence and severity of PA. An important question we wished to address “Can the cord serum levels of LDH, AST, and ALT determine babies at risk of hypoxic-ischemic encephalopathy, an important indicator of multiorgan damage?”

Patients

This was a prospective study and performed at the labor and neonatal wards of the Wesley Guild Hospital (WGH), Ilesa, a unit of the Obafemi Awolowo University Teaching Hospital in Osun State, Nigeria, from October 2014 to May 2015.

Sample Size Determination

The minimum sample size was determined using the formula for calculating the sample size for comparing a continuous variable between two groups.

The minimum number (n) recruited in each group was given by the formula:

\[
2(Z_{\alpha} + Z_{\beta})^2 (\bar{a}_1 + \bar{a}_2)^2 \left( \frac{1}{n_1 - n_2} \right)^2
\]

Patients

Consecutive term neonates delivered at the labor ward of the WGH were assessed for PA. Babies with 1-minute Apgar score less than 7 were recruited into the study as cases, and an
equal number of apparently healthy, sex-, and age-matched babies with Apgar scores of 7 and above at 1 minute of life as control.

Severe PA was taken as Apgar score less than or equal to 3 while moderate was Apgar score of 4 to 6 at 1 minute of life.4,18 Babies with obvious congenital malformation or neuromuscular abnormalities, those whose mothers were given sedative drugs, such as diazepam or magnesium sulfate, within 4 hours prior to delivery, and those whose mothers had prolonged rupture of membranes or features of chorioamnionitis were excluded from the study.

Methods

1. Five mL of blood was collected into a plain bottle from the free-flowing blood from the umbilical stump on the placental side immediately after the baby was separated from the mother. The blood was separated immediately after collection, for the estimation of serum LDH, AST, and ALT. Assays of the enzymes (LDH, AST, and ALT) were performed using ultraviolet spectrophotometer. Babies with PA were admitted into the special care baby unit of the hospital.

2. Detailed clinical and neurological evaluations were conducted after resuscitation and every 6 hours thereafter, until 48 hours of life by the investigator and the other resident doctors in the newborn unit for signs of HIE. HIE was graded as mild, moderate, and severe, using the Levene grading of HIE.19

The Levene Grading of Hypoxic–Ischemic Encephalopathy

The level of encephalopathy was assigned based on which signs predominated (Table 1).

Ethical Consideration

An Ethical Clearance Certificate was obtained from the Ethical Committee of the Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC), Ile-Ife with a protocol number ERC/2013/07/05. An informed consent was obtained from the mother of each baby.

Data Analysis

The data were analyzed using Statistical Package for the Social Sciences (SPSS) for Windows, version 17.0 (SPSS Inc; Chicago, Illinois, United States). 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, severity of PA, grades of HIE, and outcome of hospitalization. Means were compared using student’s t-test. Spearman correlation coefficient (r) was used to assess the relationship between the serum levels of LDH, AST, ALT, and the Apgar scores. Proportions and ratios were compared using Pearson’s Chi-squared (χ²) and Fisher’s exact test as appropriate. Statistically, significant level was set at p value less than 0.05 in two-tailed tests.

Results

A total of 140 babies were recruited during the study period. They comprised 70 babies with PA and 70 controls. Among the babies with PA, 38 (54.3%) were males giving a male:female ratio of 1.2:1, same as the male:female ratio of the controls.

Gestational Age

The mean gestation ages (SD) of the neonates with PA and controls were 39.2 (1.0) weeks and 39.3 (1.0). The difference was not statistically significant (p = 0.613).

Birth Weight

The mean birth weight (SD) of newborns with asphyxia was 3.2 (0.36) kg as compared with 3.2 (0.33) kg in the control group. The difference in the mean birth weights of the two groups was not statistically significant (p = 0.699).

Severity of Perinatal Asphyxia

Among the 70 babies with PA, 36 (51.4%) had severe PA with Apgar score of 0 to 3, while 34 (48.6%) had moderate PA with Apgar score of 4 to 6 at 1 minute of life. None of the 34 babies with moderate PA developed HIE, while 15 (41.7%) of the 36 babies with severe PA had HIE. Two of the babies with HIE had a mild form, five had moderate and eight had severe HIE.

Cord Blood LDH, AST, and ALT

Table 2 compares the means of cord serum levels of LDH, AST, and ALT between patients and controls. The cord levels of enzymes in the two groups were significantly different (p < 0.05) (Table 2).

Table 1 The Levene grading of hypoxic-ischemic encephalopathy

<table>
<thead>
<tr>
<th>Features</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Consciousness</td>
<td>Irritable</td>
<td>Lethargic</td>
<td>Coma</td>
</tr>
<tr>
<td>Tone</td>
<td>Hypotonia</td>
<td>Moderate hypotonia</td>
<td>Severe hypotonia</td>
</tr>
<tr>
<td>Seizures</td>
<td>No</td>
<td>Yes</td>
<td>Prolonged</td>
</tr>
<tr>
<td>Sucking/respiration</td>
<td>Poor suck</td>
<td>No suck</td>
<td>No spontaneous respiration</td>
</tr>
</tbody>
</table>

Table 2 Comparison of the means of cord serum levels of LDH, AST, and ALT between patients and the controls

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Mean (SD) U/L</th>
<th>t-Value</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDH</td>
<td>Patients</td>
<td>Controls</td>
<td></td>
</tr>
<tr>
<td></td>
<td>336.5 (142.0)</td>
<td>182.4 (54.4)</td>
<td>8.48</td>
</tr>
<tr>
<td>AST</td>
<td>45.6 (37.9)</td>
<td>26.6 (7.9)</td>
<td>4.13</td>
</tr>
<tr>
<td>ALT</td>
<td>22.2 (13.7)</td>
<td>11.4 (9.6)</td>
<td>5.40</td>
</tr>
</tbody>
</table>

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; SD, standard deviation.
serum values of LDH in babies with PA ranged from 144.4 to 688.0 U/L compared with 64.2 to 324.0 U/L in the controls; AST values ranged from 14.4 to 217.0 U/L in babies with PA compared with 13.4 to 48 U/L in the controls, while the values of ALT ranged from 2.1 to 69.0 U/L in babies with PA compared with 2.1 to 57.0 U/L in the controls. The mean (SD) serum levels of LDH, AST, and ALT were significantly higher in the cord sera of patients than the controls \( (p = 0.000) \).

- **Table 3** compares the means of cord serum levels of LDH, AST, and ALT between babies with moderate and severe PA. The means of the serum levels of the enzymes in neonates with severe PA were higher than those with moderate PA, and the differences were statistically significant.

- **Table 4** shows the comparisons of the means of the serum levels of LDH, AST, and ALT between babies with moderate and severe PA. The means of the levels of the three enzymes were statistically significantly higher in babies with HIE when compared with babies without HIE in cord blood.

- **Fig. 1** compares the cord blood levels of these enzymes in babies with HIE and those without HIE. These figures show the box-and-whisker plot of LDH, AST, and ALT in cord blood for 15 babies with HIE and 55 babies without HIE among the 70 babies with PA. The box-and-whisker plots clearly show that median values and two SDs for babies without HIE were clearly below those of babies with HIE.

- **Table 5** shows the relationship between the serum levels of LDH, AST, ALT, and severity of HIE. The means of levels of LDH, AST, and ALT were highest in cord serum of babies with severe HIE followed by those in babies with moderate HIE and lowest in those with mild HIE.

### Discussion

This study has provided data on the cord serum values of LDH, AST, and ALT in apparently healthy Nigerian newborns and Nigerian newborns with varying severity of PA. An attempt was made to evaluate the relationship between cord serum levels of LDH, AST, and ALT and severity of PA, and whether the levels could be used to identify babies at risk of early serious complications that may compromise their immediate survival.

The means of cord serum values of LDH, AST, and ALT in babies with PA were significantly higher when compared with babies without PA. This was in keeping with the findings of Karlsson et al\(^20\) and Sánchez-Nava et al.\(^21\) The cord serum values of the enzymes in babies with PA might suggest subtle leakages of intracellular enzymes, including LDH, AST, and ALT, in response to hypoxic-ischemic injury even before the delivery of the baby. This suggests that elevation of these enzymes in the serum of babies may be used in retrospective evaluation for the presence of PA in babies delivered in the absence of skilled personnel and in whom the Apgar scores are not documented.

Routine estimation of the cord serum levels of the enzymes will help in selecting babies for early and appropriate management to improve the intact survival in babies with severe PA. In this study, the cord serum levels of the enzymes increased with increasing severity of PA assessed by the Apgar score. This was similar to the results of the work done by Islam et al in 2010.\(^15\) This was likely due to the fact that with increasing severity of hypoxic-ischemic injury, more of the enzymes were being released from the damaged cells. In contrary, Choudhary et al\(^22\) reported no correlation between the levels of the enzymes and severity of PA using the Apgar scores. The differences in the findings of the different workers may partly be due to interobserver differences in the Apgar scoring. This study showed that the cord serum values of these enzymes were correlated with the severity of the hypoxic-ischemic insult.

The means of cord serum values of LDH, AST, and ALT of babies with HIE were significantly higher than the values in babies without HIE. Karunatilaka et al.\(^23\) as well as Choudhary et al.\(^22\) have shown similar relationship between the levels of enzymes and HIE, with cord serum levels of all the enzymes increased with increasing severity of HIE.

### Table 3

Comparison of the means of cord serum levels of LDH, AST, and ALT between babies with severe and moderate severe PA

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Severe mean (SD) U/L ((n = 36))</th>
<th>Moderate mean (SD) U/L ((n = 34))</th>
<th>t-Value</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDH</td>
<td>375.1 (156.6)</td>
<td>295.6 (113.2)</td>
<td>2.423</td>
<td>0.018</td>
</tr>
<tr>
<td>AST</td>
<td>61.8 (47.1)</td>
<td>28.5 (7.7)</td>
<td>4.072</td>
<td>0.000</td>
</tr>
<tr>
<td>ALT</td>
<td>27.2 (16.2)</td>
<td>16.9 (7.4)</td>
<td>3.415</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; PA, perinatal asphyxia; SD, standard deviation.

### Table 4

Comparisons of the means of levels of cord serum LDH, AST, ALT in neonates with HIE and those without HIE among babies with severe PA

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Babies with HIE ((n = 15)) Mean (SD) U/L</th>
<th>Babies without HIE ((n = 21)) Mean (SD) U/L</th>
<th>t-Value</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDH</td>
<td>476.4 (140.5)</td>
<td>302.8 (126.0)</td>
<td>3.886</td>
<td>0.000</td>
</tr>
<tr>
<td>AST</td>
<td>90.8 (57.7)</td>
<td>41.1 (21.6)</td>
<td>3.619</td>
<td>0.001</td>
</tr>
<tr>
<td>ALT</td>
<td>40.1 (15.6)</td>
<td>18.1 (8.7)</td>
<td>5.405</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; HIE, hypoxic-ischemic encephalopathy; LDH, lactate dehydrogenase; PA, perinatal asphyxia; SD, standard deviation.
HIE is sequelae of more severe hypoxic–ischemic injury, which has been found to be associated with varying levels of multiorgan dysfunction. Most of the effects of the multiple organ involvements usually go unnoticed because they are transient and often resolve without sequelae. The cord serum values of enzymes showed almost a perfect bimodal distribution with babies with and without HIE. The routine estimation of cord serum levels of the enzymes will aid clinical decision making as to which neonates with PA will likely need neuroprotective interventions. This ultimately will result in earlier appropriate management, thereby improving the prognosis.

**Conclusion**

Routine estimation of the cord serum levels of LDH, AST, and ALT will be useful as a tool for early identification of neonates with severe PA requiring prompt and appropriate management. Even when birth circumstances are not well-known and the births are not supervised by skilled personnel, the serum may assist in proper evaluation of the severity of PA and risk of HIE.

**Strengths and Weaknesses of the Study**

The babies were assessed at birth and the Apgar scores were objectively assessed. Cord sera values of the enzymes were compared between babies with and without PA. Enzyme assay was done using standardized method. A limitation of the study was that blood gas analysis was not available in the

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**Table 5** Means of cord serum levels of LDH, AST, and ALT in relation to severity of HIE

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Mild HIE</th>
<th>Moderate HIE</th>
<th>Severe HIE</th>
<th>F-Value</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDH</td>
<td>362.9 (70.6)</td>
<td>480.0 (139.5)</td>
<td>502.5 (152.2)</td>
<td>9.076</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>AST</td>
<td>52.5 (23.3)</td>
<td>61.2 (13.4)</td>
<td>118.8 (67.4)</td>
<td>25.366</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>ALT</td>
<td>31.4 (10.9)</td>
<td>36.5 (4.7)</td>
<td>46.4 (16.3)</td>
<td>25.495</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; HIE, hypoxic–ischemic encephalopathy; SD, standard deviation.
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center where the study was performed and would have been a more objective method for the assessment of PA.

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