Evaluation of Serum Creatine Kinase Muscle-Brain Fraction (CK-MB) and Lactate Dehydrogenase (LDH) as Markers of Perinatal Asphyxia in Term Neonates

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ABSTRACT
Aim and Objectives: Perinatal asphyxia contributes significantly to neonatal morbidity and mortality. This study was conducted to compare the serum levels of creatine kinase muscle-brain fraction (CK-MB) and lactate dehydrogenase (LDH) among asphyxiated and non asphyxiated term neonates and to ascertain whether these enzymes can identify asphyxiated neonates who will be at high risk for hypoxic ischemic encephalopathy and multi-organ dysfunction.

Material and Methods: Prospective observational study. A study was conducted on 50 neonates comprising the cases and 50 neonates comprising the controls meeting the inclusion and exclusion criteria. The blood samples for CK-MB and LDH was drawn at 8±2 and 72±2 hours of age respectively and sent for analysis. A serum CK-MB value >92.6 U/L at 8 hours and LDH value >580 U/L at 72 hours was taken as the cut-off level.

Results: The cut-off CK-MB value of >92.6 U/L has 28% sensitivity with a specificity of 100%. CK-MB has a positive predictive value of 100% with a negative predictive value of 58.14%. The cut-off LDH value of >580 U/L has 59.18% sensitivity with a specificity of 92%. LDH has a positive predictive value of 87.88% with a negative predictive value of 69.70%.

Conclusion: Estimation of CK-MB at 8 hours of life and LDH at 72 hours of life can help distinguish an asphyxiated from a non-asphyxiated term neonate in correlation with history and clinical features in the neonate.

KEYWORDS: Perinatal asphyxia, Creatine kinase muscle-brain fraction (CK-MB), Lactate dehydrogenase (LDH), hypoxic ischemic encephalopathy (HIE)

INTRODUCTION
Globally, hypoxia of the newborn (birth asphyxia) or the fetus (“fresh stillbirth”) is estimated to account for 23% of the 4 million neonatal deaths and 26% of the 3.2 million stillbirths each year [1]. Data from National Neonatal Perinatal database (NNPD) suggests that perinatal asphyxia contributes to almost 20% of neonatal deaths in India [2]. In India, 8.4% of inborn babies have a one minute Apgar score less than 7 and 1.4% suffer from hypoxic ischemic encephalopathy (HIE) [2]. Many different assessments attempt to predict fetal well-being during labour and following delivery. These include observing for the passage of meconium, electronic fetal heart rate monitoring via a cardiotocograph, Apgar score and the assessment of fetal acid-base balance. The signs of asphyxial injury are nonspecific and overlap with other illnesses. It is difficult to retrospectively diagnose perinatal asphyxia in the absence of perinatal records.

Transient myocardial ischemia (TMI) with myocardial dysfunction may occur in any neonate with a history of perinatal asphyxia. An elevated serum creatine kinase muscle-brain fraction (CK-MB) fraction or cardiac troponin T (cTnT) level may be helpful in determining the presence of myocardial damage. An elevation of serum CK-MB fraction of >5% to 10% may indicate myocardial injury [3]. Leakage of intracellular enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) signaling multi organ dysfunction is seen together with HIE after perinatal asphyxia [4]. We conducted this study to ascertain whether common enzyme assays can distinguish an asphyxiated from a non asphyxiated neonate.
MATERIALS AND METHODS

The study was a prospective study conducted on asphyxiated and non-asphyxiated term neonates recruited from Neonatal Intensive Care Unit (NICU) and Post natal wards of Sree Mookambika Institute of Medical Sciences, Kulasekharam, Kanyakumari from September 2012 to December 2013. Cases and Controls comprised of asphyxiated and non-asphyxiated babies, respectively.

Case group: Includes 50 neonates fulfilling the following criteria

**Inclusion criteria**
1. Gestational age ≥ 37 weeks
2. Appropriate for gestational age.
3. The neonates who were identified to have experienced perinatal asphyxia when at least 3 of the following were present:
   A) Intrapartum signs of fetal distress, as indicated by non reassuring Non-Stress Test (NST) on continuous electronic fetal monitoring and/or by meconium staining of the amniotic fluid.
   B) Apgar score of <7 at 1 minute of life.
   C) Requirement of positive pressure ventilation for >1 minute.
   D) Profound metabolic or mixed acidemia (pH<7.00) in an umbilical artery blood sample, if obtained.
   E) Mild, moderate or severe hypoxic ischemic encephalopathy (HIE), as defined by Levene MI [5].

Exclusion criteria
3. Neonates born to mothers who would had received magnesium sulphate within 4 hours prior to delivery or opioids (pharmacological depression).
4. Hemolytic disease of the newborn.

Control group: Includes 50 term apparently healthy neonates appropriate for gestational age without signs of perinatal asphyxia as evidenced by normal fetal heart rate patterns, clear liquor and one minute Apgar score ≥7.

Detailed maternal history, assessment of intrauterine fetal well being by continuous electronic fetal monitoring, meconium staining of amniotic fluid, birth events, Apgar score, sex of the baby and weight of the baby were recorded on the precoded proforma. Gestational age was assessed from last menstrual period and New Ballard score. Arterial blood gas analysis (ABG) was done if umbilical arterial blood was obtained. Thorough clinical and neurological examination was done for all the neonates included in the study. The asphyxiated neonates (case group) were monitored for seizures, hypotonia and HIE in the immediate neonatal period in the NICU. A clinical grading system by Levene MI (Table-1) [5] was used to grade the severity of HIE.

The cases were also observed for other systemic effects of asphyxia. Blood sample were collected from the neonates and sent for CK-MB and LDH levels. Blood for CK-MB was drawn at 8±2 hours. Blood for LDH was drawn at 72±2 hours of age. [6,7]. Serum CK-MB was analyzed by Immuno-inhibition method on 1 mL clotted blood. LDH level analysis was done by German Society for Clinical Chemistry (DGKC) method on 1 mL clotted blood. The upper limit of the normal range of CK-MB at 5-8 hours of life is 7.9% of 1.175 U/L which is ~92.6 U/L (8). A serum CK-MB value >92.6 U/L at 8 hours was taken as the cut-off level. The normal reference value of LDH in neonates and infants <1 year is 170-580 U/L (8). A value >580 U/L at 72 hours was taken as the cut-off level.

The case group also had other investigations and imaging studies done as required for post-resuscitation management of asphyxiated neonates. The causes for hypotonia, seizures, lethargy, poor feeding other than HIE were ruled out with relevant investigations available. Peripheral smear for erythrocyte morphology and reticulocyte count was used to document hemolytic disease of the newborn.

Statistical analysis

Descriptive statistical analyses were carried out. Student t test (two tailed, independent) was used to find the significance of study parameters on continuous scale between two groups. Chi-square/ Fisher Exact test was used to find the significance of study parameters on categorical scale between two groups. Sensitivity, specificity and predictive values of the tests were calculated. ROC Curve analysis was performed to find the diagnostic performance of CK-MB and LDH.

RESULTS

Cases (n=50) and controls (n=50) had similar rates of male sex (60% vs. 58%), mean birth weight (2.94±0.33 kg vs. 3.04±0.33 kg) and primi gravida mothers (66% vs. 58%). 39 (78%) cases had Non Reassuring NST and 32 (64%) cases were born through meconium stained amniotic fluid. Incidence of cesarean section and instrumental delivery were significantly more in case group (88%) compared to control group (34%) with P<0.001. 8 (16%) cases had Apgar score ≤7 at 5 min and 19 (38%) cases had abnormal neurological examination. Among the 50 neonates in the case group, 3 (6%) had mild HIE, 12 (24%) had moderate HIE and 4 (8%) had severe HIE during the course in NICU. There was no significant correlation between severity of HIE and Apgar scores of 0-3 and 4-6 at 1 minute (P=0.158) and at 5 minute (P=0.421). Comparison of CK-MB and LDH levels in the 2 groups are shown in Table-2 and 3. The sensitivity, specificity and predictive values of CK-MB and LDH are depicted in Table-4.
Table: 1 Clinical grading system for hypoxic-ischaemic encephalopathy by Levene MI (5)

<table>
<thead>
<tr>
<th>Feature</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Consciousness</td>
<td>Irritable</td>
<td>Lethargy</td>
<td>Comatose</td>
</tr>
<tr>
<td>Tone</td>
<td>Hypotonia</td>
<td>Marked 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>Unable to suck</td>
<td>Unable to sustain Spontaneous respiration</td>
</tr>
</tbody>
</table>

Table: 2 Shows comparison of cut-off levels of CK-MB and LDH in cases and controls.

<table>
<thead>
<tr>
<th>Markers</th>
<th>Cases (n=50)</th>
<th>Control (n=50)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK-MB (cut-off 92.6 U/L)</td>
<td>50</td>
<td>50</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>i. &lt;92.6 U/L</td>
<td>36(72.0%)</td>
<td>50(100.0%)</td>
<td></td>
</tr>
<tr>
<td>ii. &gt;92.6 U/L</td>
<td>14(28.0%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>LDH (cut-off 580 U/L)</td>
<td>49*</td>
<td>50</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>i. &lt;580 U/L</td>
<td>20(40.82%)</td>
<td>46(92.0%)</td>
<td></td>
</tr>
<tr>
<td>ii. &gt;580 U/L</td>
<td>29(59.18%)</td>
<td>4(8%)</td>
<td></td>
</tr>
</tbody>
</table>

(*1 neonate with severe HIE died on day 2 of life because of which LDH could not be estimated at 72 hours of life.)

Table: 3 Shows comparison of CK-MB and LDH levels in cases and controls.

<table>
<thead>
<tr>
<th>Markers</th>
<th>Cases</th>
<th>Controls</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK-MB (U/L) (at 8 hours)</td>
<td>50</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>MEAN±SD</td>
<td>83.98±19.60</td>
<td>44.56±14.53</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Median (range)</td>
<td>82 (48-142)</td>
<td>42.5 (18-74)</td>
<td></td>
</tr>
<tr>
<td>LDH (U/L) (at 72 hours)</td>
<td>49</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>MEAN±SD</td>
<td>555.65±105.95</td>
<td>402.78±85.24</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Median (range)</td>
<td>563 (154-881)</td>
<td>402 (116-602)</td>
<td></td>
</tr>
</tbody>
</table>

The cut-off CK-MB value of >92.6 U/L has 28% sensitivity with a specificity of 100%. CK-MB has a positive predictive value of 100% with a negative predictive value of 58.14%. The cut-off LDH value of >580 U/L has 59.18% sensitivity with a specificity of 92%. LDH has a positive predictive value of 87.88% with a negative predictive value of 69.70%.

The diagnostic performance of LDH is better than CK-MB (Figure-1). The correlation of cut-off CK-MB level of 92.6 U/L with the severity of HIE was not significant (P=0.569). The correlation of cut-off LDH level of 580 U/L with the severity of HIE was also not significant (P=0.501).

Table: 4. Shows sensitivity, specificity and predictive values of CK-MB and LDH.

<table>
<thead>
<tr>
<th>Markers</th>
<th>Cut-off</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>Area under curve</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK-MB (U/L) (at 8 hours)</td>
<td>&gt;92.6</td>
<td>28.00%</td>
<td>100.0%</td>
<td>100.0%</td>
<td>58.14%</td>
<td>0.921</td>
<td>Excellent</td>
</tr>
<tr>
<td>LDH (U/L) (at 72 hours)</td>
<td>&gt;580</td>
<td>59.18%</td>
<td>92.0%</td>
<td>87.88%</td>
<td>69.70%</td>
<td>0.954</td>
<td>Excellent</td>
</tr>
</tbody>
</table>
DISCUSSION

Several studies have been conducted to evaluate better markers that help to differentiate asphyxial and non-asphyxial etiology in neonates. Primhak et al [6] observed that the CK-MB in both normal (n=43) and asphyxiated (n=20) neonates, peaked at 8 hours and fell by 72 hours. Absolute and percentage CK-MB levels were higher in asphyxiated babies.

Omokhodion SI et al [9] studied the creatine kinase (CK) and CK-MB activities in 23 perinatally asphyxiated newborns and 12 healthy controls during the first 100 h of life. The asphyxiated infants had significantly elevated mean CK and absolute CK-MB but no fractional CK-MB activities. The healthy controls, on the other hand, showed a steady decline in the activities of these enzymes from birth. Fonseca E et al [10] found that antepartum fetal distress is associated with release of CK-BB, and particularly CK-MB; therefore, these biochemical markers may indicate either brain or myocardial damage. Barberi et al [11] reported that CK, CK-MB, CK-MB/CK ratio and LDH were all increased in an asphyxiated group, while in a group with respiratory distress; only CK-MB and the CK-MB/CK ratio were abnormal.

The study by Karunatilaka DH et al [12] also concluded that both the CK and LDH values are raised in birth asphyxia. LDH had 100% sensitivity, while CK-MB had 100% specificity for asphyxia in a study by Reddy S et al [13]. Rajakumar PS et al [14] observed that the cardiac enzymes, cTnT and CK-MB, were significantly elevated in cases when compared with controls. In 2010, Karlsson M et al [15] in their clinical and experimental study done in 2008 on evaluation of organ damage in perinatal asphyxia concluded that in asphyxiated infants with differing degree of HIE and in infants where there had been signs of fetal distress during birth a cut off level of 1049 U/L for LDH was the most suitable predictor of mild, moderate, and severe HIE with a sensitivity of 100% and specificity of 97%. This study shows that estimation of CK-MB at 8 hours of life and LDH at 72 hours of life can help distinguish an asphyxiated from a non-asphyxiated term neonate with reasonable degree of accuracy. LDH is having more diagnostic value than CK-MB with more Area under ROC value when compared to CK-MB (0.954 vs. 0.921), but both are excellent tests to differentiate asphyxiated and non-asphyxiated term neonates.

CONCLUSION

Estimation of CK-MB at 8 hours of life and LDH at 72 hours of life can help distinguish an asphyxiated from a non-asphyxiated term neonate in correlation with history and clinical features in the neonate.
REFERENCES


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