Study to Determine Correlation of Elevated Serum Creatine Kinase-Muscle Brain Fraction (CK-MB) and Lactate Dehydrogenase (LDH) Levels with Asphyxia Neonatorum and Severity of Hypoxic Ischaemic Encephalopathy

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Abstract

Background: Asphyxia Neonatorum is a significant contributor to neonatal mortality and morbidity. Its burden in resource-poor countries prompts the development of investigative modalities that offers accuracy and affordability.

Objective: To determine that serum CK-MB and LDH estimation could be markers of Asphyxia neonatorum, and the elevated levels directly correlate with the grade of severity of Hypoxic Ischemic Encephalopathy (HIE) as defined by Levine.

Methods: In this single-center, non-interventional study, we included 100 asphyxiated neonates from level III NICU in the case group, and 100 apparently healthy neonates from the post-natal ward admitted over 18 months at RCSM Government Medical College and CPR Hospital, Kolhapur. 100 healthy neonates were the control group.

Results: The median 8-hr serum CK-MB in the case group was 74 U/L and in the control group as 63.5 U/L with P<0.001. Whereas the median serum LDH level in the case group was 597 U/L and the control group was 383.5 U/L with P<0.001. Raised LDH (cut off 580 U/L) had 100% specificity, while CK-MB (cut off 92.6 U/L) had 100% specificity for asphyxia. Amongst the neonates with HIE (n=60), 56% had raised CK-MB (cut off 92.6) and 80% had raised LDH (cut off 580 U/L). 100% of neonates with HIE Grade III (n=9) had elevated CK-MB and LDH levels.

Conclusion: The present study concluded that CK-MB and LDH as biochemical markers can be utilitarian in the diagnosis of perinatal asphyxia.
Introduction

Perinatal asphyxia is marked by the presence of hypoxia, hypercarbia, and acidosis due to impaired fetal blood gas exchange in the first and second stages of labor. In India, 20% of all neonatal deaths occur due to birth asphyxia [1]. Whereas in low-income countries, 70% of all stillbirths can be attributed to perinatal asphyxia [2]. 8.4% of babies have a 1 min APGAR score less than 7 whereas 1.4% were diagnosed with hypoxic-ischemic encephalopathy (HIE) in India [3]. About 28.8% of asphyxiated neonates die, due to hypoxic complications of perinatal asphyxia [4].

Hypoxic intrapartum event and subsequent ischemic-reperfusion injury in asphyxia cause multi-systemic damage and render neonate at risk of developing hypoxic-ischemic encephalopathy, frequent bradycardia, transient myocardial ischemia, arrhythmias, pulmonary hypertension, renal failure. Myocardial dysfunction results in an elevated serum creatine kinase muscle-brain fraction (CK-MB). Whereas leakage of intracellular enzymes following hypoxic cell death, results in elevated levels of lactate dehydrogenase (LDH).

The clinical presentation of asphyxia overlaps with other common neonatal diseases. Estimation of CK-MB and LDH enzymes can prove instrumental in establishing a retrospective diagnosis of perinatal asphyxia when antenatal history remains obscured. The study aims to also prove that elevated enzyme levels correlate directly with the grade of HIE.

Methods

In this single-center, non-interventional study, we included 100 asphyxiated neonates from level III NICU in the case group, and 100 apparently healthy neonates from the post-natal ward admitted over 18 months at RCSM Government Medical College and CPR Hospital, Kolhapur.

Inclusion criteria

- Gestational age > 37 weeks,
- Birth weight must be appropriate for gestational age,
- And signs of perinatal asphyxia suggested by at least 3 out of the following 5:
  1. Non-reassuring NST or Meconium-stained amniotic fluid suggesting Intrapartum distress.
  2. APGAR Score < 7 at 1 min of life.
  3. Neonatal Resuscitation requiring Positive Pressure Ventilation > 1 min.
  4. Metabolic acidemia i.e., pH< 7 in umbilical arterial blood samples.
  5. Mild, moderate, or severe HIE.

Exclusion criteria

Presence of congenital malformation,
Maternal history of drug abuse,
Neonates were born to mothers who received magnesium sulfate within 4 hours prior to delivery.
100 healthy neonates appropriate for gestational age, with normal fetal heart patterns, normal antenatal fetal assessment, and 1 min APGAR score ≥7 were recruited for the control group. Due permission from the Institutional Ethics Committee was taken and written informed consent from the patient’s parents was obtained before enrolling in the study.

The neonates enrolled in the study underwent clinical and neurological examinations. Case group neonates admitted to NICU were under consistent monitoring for deterioration in neurological and hemodynamic status. Venous blood samples by peripheral venepuncture were obtained for serum assessment of the following enzyme levels: Creatine Kinase-Muscle Brain Fraction. (CK-MB) at 8±3 hours of life.[4-6] Lactate Dehydrogenase (LDH) at 72±3 hours of life [5-7]. Cut-off values were taken as CK-MB value >92.6 U/L at 8 hours, and LDH value >580 U/L at 72 hours [8].

Statistical method
Descriptive statistical analysis was used. Results are presented as Mean ± SD (Minimum-Maximum). The study parameter’s significance was determined by the student t-test on a continuous scale, whereas for categorical scale amongst multiple groups Chi-Square test was used. Assessment of significance is determined at a 5% level of significance. The diagnostic accuracy of CK-MB and LDH was determined by Receiver Operating Characteristic Curve (ROC) assessment. Negative Predictive Value and Positive Predictive values (NPV AND PPV), as well as Specificity and Sensitivity of enzyme estimation, were calculated.

Results
Cases (n=100) and Control (n=100) groups had comparable gender distribution with 58% Vs 48% of male preponderance, multigravida mothers of 38% Vs 57%, and mean birth weight was 3.19±0.41 kg Vs 3.09±0.36 kg. The incidence of LSCS was 31% Vs 57%, more in the control group and instrumental delivery was 6% Vs 0% more in the case group. In the case of the group, about 70% had non-reassuring NST, 74% had Meconium-stained Amniotic fluid, at 1 min 89% had APGAR score <3, and 11% had APGAR Score 4-6, at 5 min 33% had APGAR score 4-7, and 67% had APGAR score >7.

Neurological examination of the case group revealed 40% had hypotonia and 6% were flaccid, and 35% had seizures. 26%, 27%, and 9% were in mild, moderate, and severe stages of HIE. Complications amongst the case group showed respiratory distress in 64%, HIE in 62%, shock in 15%, acute kidney injury in 3%, and congestive cardiac failure in 2%. 11% of case group neonates expired.

Table 1 depicts the comparison of creatine CK-MB Fraction and Lactate Dehydrogenase (LDH) levels amongst the case and control groups. Table 2 exhibits the diagnostic Accuracy parameters of CK-MB and LDH estimation in Perinatal Asphyxia.
Table 1: Comparison of CK-MB and LDH levels amongst case and control group

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cases (n=100)</th>
<th>Control (n=100)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK-MB Levels</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;92.6 IU/L</td>
<td>66%</td>
<td>100%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>≥92.6 IU/L</td>
<td>34%</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>LDH Levels</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;580 IU/L</td>
<td>49%</td>
<td>100%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>≥580 IU/L</td>
<td>51%</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>CK-MB (U/L) at 8 hours</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>84.80 ± 28.30</td>
<td>63.10 ± 14.00</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Median (Range)</td>
<td>74.0 (49-175)</td>
<td>63.5 (29-91)</td>
<td></td>
</tr>
<tr>
<td>LDH (U/L) at 72 hours</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>544.71 ± 180.17</td>
<td>568.00 ± 114.40</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Median (Range)</td>
<td>597.0 (122-892)</td>
<td>593.5 (118-563)</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Diagnostic accuracy parameters of CK-MB and LDH estimation

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>Area under ROC Curve</th>
</tr>
</thead>
<tbody>
<tr>
<td>CKMB Levels (Cut-off 92.6 IU/L)</td>
<td>34%</td>
<td>100%</td>
<td>100%</td>
<td></td>
<td>60.24%</td>
</tr>
<tr>
<td>LDH Levels (Cut-off 580 IU/L)</td>
<td>51%</td>
<td>100%</td>
<td>100%</td>
<td></td>
<td>67.11%</td>
</tr>
</tbody>
</table>

Figure 1 shows the receiver operator characteristic curve of CK-MB and LDH levels. The area under the curve for CK-MB at 8 hrs was 0.803 and LDH at 72 hrs was 0.856. Figure 2 demonstrates a correlation between CK-MB and LDH levels with the severity of HIE grade in asphyxiated neonates.

Figure 1: Receiver Operator Characteristics (ROC) curves of enzyme estimation

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Content is available online at https://erwejournal.com/
Figure 2: Correlation of CK-MB and LDH cut-off values with HIE grade in asphyxiated neonates

Among the neonates with HIE (n=60) having CK-MB value >92.6 U/L, 15.00% had HIE Stage I, 26.67% had HIE Stage II and 15.00% had HIE Stage III. Of neonates with HIE (n=60) with LDH value >580 U/L, 25.00% had HIE Stage I, 40.00% had HIE Stage II, and 15.00% had HIE Stage III. All HIE Stage III (100%) had CK-MB levels > 92U/L and LDH levels >580 U/L. Elevated CK-MB and LDH levels correlate directly with the severity of HIE Stages.

Discussion

This study determines that CK-MB and LDH levels are a tool for differentiating asphyxiated neonates from non-asphyxiated neonates. Under circumstances of unknown perinatal history and non-specific clinical presentation of perinatal asphyxia, estimation of CK-MB and LDH levels prove instrumental in establishing the diagnosis.

Elevated levels of serum CK-MB and LDH levels in asphyxiated neonates demonstrated in our study are also supported by the conclusion of the following studies: Primhak et al demonstrated that the CK-MB enzyme levels were elevated among asphyxiated babies. CK-MB peaked at 8 hours [5]. Sanchez-Nava et al demonstrated increased levels of LDH asphyxiated babies [9]. Omokhodion SI et al showed elevated mean CK and absolute CK-MB levels in neonates with asphyxia [10]. Barberi et al study revealed elevated levels of CK, CK-MB, LDH, and CK-MB: LDH ratio, amongst neonates of the asphyxiated group [11]. Karunatilaka et al, showed that CK-MB and LDH values are elevated in neonates with asphyxia [12].

Our study revealed a strong correlation between the severity of Hypoxic Ischemic Encephalopathy, complications of asphyxia, and elevated levels of CK-MB and LDH Levels. As also suggested by; Boo NY et al who reported that higher concentrations of cTnT and CK-MB levels were observed amongst neonates with asphyxia vs controls [13].
Similar observations were confirmed by the study conducted by Rajakumar PS et al. C H Rajeesha et al, in her study on asphyxiated neonates concluded that Serum LDH value showed maximum values between 18 and 24 hours of life. Noticed a strong correlation between serum LDH levels and higher stages of HIE [14]. AI Munteanu et al implied that LDH is a good predictor of HIE in the first 12/24 h after birth [15].

**Conclusion**

The current study concludes that CK-MB and LDH as biochemical markers can be utilitarian in the diagnosis of perinatal asphyxia. Serum LDH levels at 72 hours have better diagnostic accuracy than serum CK-MB levels at 8 hours with more area under the ROC curve, in establishing retrospective diagnosis of Perinatal asphyxia. Elevated CK-MB and LDH levels directly correlate with the severity of the grade of HIE determined by Levine.

**Ethical Approval:** N/A

**Conflict of Interest:** Nil

**Financial Disclosure:** None

**References**


