Evaluation of Lactate Dehydrogenase, Creatine Kinase and Hepatic Enzymes for the Retrospective Diagnosis of Perinatal Asphyxia Among Sick Neonates

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ABSTRACT

It is difficult to make a retrospective diagnosis of perinatal asphyxia in symptomatic neonates delivered non-institutionally. We studied serum creatine kinase muscle-brain fraction (CK-MB), lactate dehydrogenase (LDH), glutamic oxaloacetic transaminase (SGOT) and glutamic pyruvate transaminase (SGPT) for differentiating asphyxiated (n=25) from non-asphyxiated (n=20) neonates who present with non-specific signs of sickness. CK-MB was assayed at 8 and 24 h; and LDH, SGOT and SGPT at 72 h of life. On comparing cases and controls, median 8-hr CK-MB [80 U/L vs. 26 U/L respectively, P<0.001], median 24-hr CK-MB [33.5 U/L vs. 21.5 U/L respectively, P=0.009] and median LDH [965 U/L vs. 168 U/L respectively, P<0.001] were higher in asphyxiated neonates. Raised LDH had 100% sensitivity, while CK-MB had 100% specificity for asphyxia. LDH had the highest area under ROC curve (0.998). We conclude that LDH at 72 hr of life is most accurate at differentiating asphyxiated from non-asphyxiated symptomatic neonates.

Key words: Creatine kinase, Lactate dehydrogenase, Newborn, Perinatal asphyxia.

INTRODUCTION

In India, 8.4% of inborn babies have a 1 minute Apgar score less than 7 and 1.4% suffer from hypoxic ischemic encephalopathy (HIE)(1). Only a third of deliveries in India are institutional(2) and many asphyxiated babies are brought late to hospitals. The signs of asphyxial injury are non-specific and overlap with other illnesses. In the absence of perinatal records, it is difficult to retrospectively diagnose perinatal asphyxia.

We conducted this study to ascertain whether common enzyme assays in neonates with non-specific sickness can distinguish an asphyxial from a non-asphyxial etiology.

METHODS

This prospective, matched cohort study was conducted on babies admitted to a level III neonatal unit. Cases and Controls comprised of asphyxiated and non-asphyxiated babies, respectively. Study subjects included 45 neonates (convenience sample) delivered at ≥33 wks of gestation with birth wt ≥1250 g; the delivery was attended by a pediatrician; and they became symptomatic within 6 hours of birth with at least 1 non-specific sign of sickness—tachypnea, chest retractions, grunt, lethargy, poor feeding, hypotonia, irritability, central cyanosis, cardiac gallop rhythm, cardiac murmur, shock and abdominal distension.

Gestation was assessed from last menstrual period, early fetal ultrasound and New Ballard score. Birth weight was recorded electronically with least count of 1 g. Babies with major malformations, those born to mothers who had received pethidine, or magnesium sulphate within 4 h prior to delivery or who had obvious signs of HIE were excluded. Subjects were enrolled after written, informed consent from a parent. The study was approved by the Institute Ethics Committee.

Cases were subjects with 1 minute Apgar score of <7 and fetal bradycardia, meconium stained liquor or cord pH <7; and without any other independent cause...
for the clinical signs. Controls were unasphyxiated neonates with normal fetal heart rate patterns, clear liquor, cord pH > 7.20 and 1 min Apgar score ≥ 7.

Maternal and neonatal details were recorded. In all subjects, serum creatine kinase muscle-brain fraction (CK-MB) was performed at 6±1 h and 24±2 h; and serum lactate dehydrogenase (LDH), serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvate transaminase (SGPT) at 72±2 h. Laboratory technicians were masked to the identity of the subjects. Serum CK-MB was analyzed by immunoassay on 1 mL clotted blood(3). A value above 92.6 U/L at 8 hours and above 60 U/L at 24 hours was abnormal(4). Serum LDH was analyzed by the liquiUV test on 1 mL clotted blood(5). A value above 580 U/L was abnormal(4). SGOT and SGPT were analyzed by standard methods on 1 mL clotted blood. Values of SGOT above 140 U/L and SGPT above 50 U/L were abnormal(4).

Baseline characteristics and clinical and laboratory data were described by descriptive statistics. Cases and Controls were compared by Mann Whitney U test for skewed numerical variables and Fisher’s exact test for categorical variables. Sensitivity, specificity and predictive values of the tests were calculated. ROC curves were generated and the areas under the curve were compared to determine the most appropriate laboratory parameter.

RESULTS

Cases (n=25) and Controls (n=20) had similar rates of prematurity (52% vs. 70%), male sex (64% vs. 90%), primi gravida mothers (52% vs. 35%), incidence of prolonged labour (0 vs. 7%) and prolonged rupture of membranes (18% vs. 36%). Significantly more Cases, as compared to Controls, were delivered by emergency cesarean section [14 (58%) vs. 3 (15%); P=0.003]. Ninety-two percent Cases had fetal bradycardia, 8% were born through meconium stained liquor and 48% cases had a cord pH < 7. Eighty-four percent Cases required ventilation by bag-and-mask, 20% intubation and 8% chest compression. Apgar score was < 7 in 48% and 10% subjects at 5 and 10 minutes of age, respectively.

Among the symptoms, “hypotonia” was commoner in the Cases than Controls [17 (68%) vs. 0; P<0.001] and vice versa for “poor feeding” [12 (48%) vs. 16 (80%); P=0.02]. There was a trend for “shock” to be more common among Cases than Controls [4 (16%) vs. 0; P=0.06]. Other symptoms were not significantly different between both groups. No subject had clinical features of Stage III HIE by Sarnat and Sarnat scoring or Grade III by Levene’s classification(6,7).

Comparative values of all enzymes in the 2 groups is shown in Table I. The sensitivity, specificity and predictive values of CK-MB and LDH are depicted in Table II. Receiver operator characteristics (ROC) curves were generated for CK-MB at 8 hours, 24 hours and LDH at 72 hours (Fig. 1). The area under curve for LDH was 0.998 [95% C.I. 0.99, 1.0 (P<0.001)]. The area under curve for CK-MB at 8 hrs was 0.82 [95% C.I. 0.69, 0.94 (P=0.01)]. The area under curve for CK-MB at 24 hrs was 0.74 [95% C.I. 0.59, 0.89 (P=0.009)].

DISCUSSION

This study showed that LDH at 72 hours of life was the most accurate test for discriminating asphyxia from other illnesses among neonates who presented with non-specific signs of illness. The strength of our design was that it resembled sick patients in real-life clinical scenarios. None of the subjects had severe grades of HIE, whose diagnosis is generally obvious. Seventeen cases had hypotonia, of which 13 also had lethargy, but there were no other features of encephalopathy. This design was unlike many previous studies that had either compared asphyxiated neonates with healthy controls; or had evaluated asphyxiated neonates, irrespective of whether they became symptomatic; or had limited their case selection only to those who had HIE.

Jedeiken(8) described that the normal levels of all iso-enzymes of CK in newborns peaked at 5-33 hours post-natally. In a study by Primhak, et al.(9), the CK-MB in both normal (n=43) and asphyxiated (n=20) neonates, peaked at 8 hours and fell by 72 hours. Absolute and percent CK-MB levels were higher in asphyxiated babies. Sanchez-Nava, et al.(10) showed that SGOT, SGPT and LDH were raised among asphyxiated babies.
**TABLE I**  COMPARISON OF LEVELS OF ENZYMES BETWEEN CASES AND CONTROLS

<table>
<thead>
<tr>
<th>Laboratory test</th>
<th>Cases N=25</th>
<th>Controls N=20</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK-MB at 8 h in U/L:</td>
<td>mean ± SD</td>
<td>176.1 ± 243</td>
<td>33 ± 20.8</td>
</tr>
<tr>
<td></td>
<td>median (range)</td>
<td>80 (13-1092)</td>
<td>26 (13-74)</td>
</tr>
<tr>
<td>CK-MB at 8 h above 92.6 U/L</td>
<td>9 (36%)</td>
<td>0 (0%)</td>
<td>0.006</td>
</tr>
<tr>
<td>CK-MB at 24 h in U/L:</td>
<td>mean ± SD</td>
<td>49.6 ± 36</td>
<td>20.8 ± 7</td>
</tr>
<tr>
<td></td>
<td>median (range)</td>
<td>33.5 (8-124)</td>
<td>21.5 (7-46)</td>
</tr>
<tr>
<td>CK-MB at 24 h above 60 U/L</td>
<td>9 (36%)</td>
<td>0 (0%)</td>
<td>0.006</td>
</tr>
<tr>
<td>LDH at 72 h in U/L:</td>
<td>mean ± SD</td>
<td>1109.5 ± 520.6</td>
<td>231.5± 177.5</td>
</tr>
<tr>
<td></td>
<td>median (range)</td>
<td>965 (595-3142)</td>
<td>168 (58-642)</td>
</tr>
<tr>
<td>LDH at 72 h above 580 U/L</td>
<td>25 (100%)</td>
<td>2 (10%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SGOT at 72 h in U/L:</td>
<td>mean ± SD</td>
<td>20.7 ± 12.5</td>
<td>18.8 ± 8</td>
</tr>
<tr>
<td></td>
<td>median (range)</td>
<td>15 (9-67)</td>
<td>17 (9-32)</td>
</tr>
<tr>
<td>SGOT at 72 h above 140 U/L</td>
<td>0</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>SGPT at 72 h in U/L:</td>
<td>mean ± SD</td>
<td>15.2 ± 23.1</td>
<td>12.7 ± 4.9</td>
</tr>
<tr>
<td></td>
<td>median (range)</td>
<td>9 (7-121)</td>
<td>11 (7-23)</td>
</tr>
<tr>
<td>SGPT at 72 h above 50 U/L</td>
<td>0</td>
<td>0</td>
<td>–</td>
</tr>
</tbody>
</table>

*CK-MB: Creatine kinase muscle brain fraction; LDH: Lactate dehydrogenase; SGOT: Serum glutamic oxaloacetic transaminase; SGPT: Serum glutamic pyruvic transaminase*

The levels of the above enzymes are also raised in other sick infants, but the magnitude of elevation is higher in asphyxiated neonates. 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. Lackmann, et al. (12) found that newborn infants with asphyxia have significantly higher values of SGOT, LDH and hydroxybutyrate compared to neonates with only RDS, and presence of RDS among asphyxiated neonates did not alter the enzyme levels.

The results of the present study would be of utility to pediatricians in referral hospitals, who receive sick neonates, whose birth details are not well recorded. LDH could be used at 3 days of age to diagnose asphyxia retrospectively in such cases. It is not possible to draw any conclusions regarding the utility of LDH before or after 72 hours. A limitation was that we did not exclude subjects with hemolysis or hepatitis who could have had an increase in LDH due to reasons other than asphyxia.

**ACKNOWLEDGMENTS**

We gratefully acknowledge the help provided by Dr Rajendra Prasad and his group in the Pediatric Biochemistry laboratory in analyzing lactate dehydrogenase.

**TABLE II**  SENSITIVITY, SPECIFICITY AND PREDICTIVE VALUES OF ENZYMES

<table>
<thead>
<tr>
<th>Laboratory test</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive predictive value</th>
<th>Negative predictive value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK-MB 8 h (cut–off 92.6 U/L)</td>
<td>36%</td>
<td>100%</td>
<td>100%</td>
<td>52%</td>
</tr>
<tr>
<td>CK-MB 24 h (cut–off 60 U/L)</td>
<td>36%</td>
<td>100%</td>
<td>100%</td>
<td>52%</td>
</tr>
<tr>
<td>LDH (cut-off 580 U/L)</td>
<td>100%</td>
<td>89%</td>
<td>92%</td>
<td>100%</td>
</tr>
</tbody>
</table>

*CK-MB: Creatine kinase muscle brain fraction; LDH: lactate dehydrogenase*
Contributors: SR collected the data and samples, SD analyzed the results and wrote the manuscript, AN provided the concept and supervised the manuscript.

Funding: None.

Competing interests: None stated.

REFERENCES


Fig 1 Comparison of receiver operator characteristics curves of the markers of asphyxia.

What This Study Adds?

- Serum lactate dehydrogenase measured at 72 hours of age is the best choice to differentiate between asphyxial from a non-asphyxial etiology of non-specific clinical signs of illness in a newborn.