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

Background: The enzymes CK-MB & LDH are used as potential predictors of timing and grade of HIE in newborns with perinatal asphyxia. **Objectives:** To differentiate HIE neonates from non-HIE ones on the basis of significant rise of LDH & CK-MB. **Methodology:** Prospective cross-sectional analytical study. Among 164 newborns, 82 comprising the cases and 82 neonates comprising the controls met the inclusion and exclusion criteria. The umbilical cord blood samples for CK-MB and LDH was drawn and sent for analysis. A serum CK-MB value >92.6 U/L and LDH value >580 U/L was taken as the cut-off level. Descriptive statistical analyses were done to find the significance between two groups. ROC Curve analysis was performed to find the diagnostic performance of CK-MB and LDH. **Results:** Out of total 164 neonates studied, 18.3% had moderate HIE & 11%had severe HIE while 56.1%had No HIE. Seventy-two newborns were found to have LDH levels >580 U/L out of which 71 were in case group and 1 was in control group. Among the 164 neonates studied, 7.9% from case group were found to have CK-MB levels >92.6 U/L. Both the results of LDH & CK-MB levels were very significant with P value <0.001. Area under ROC (Receiving operating Characteristic) value of LDH when compared to CK-MB is (0.978 vs. 0.731). **Conclusion:** Estimation of CK-MB and LDH enzymes can help to distinguish asphyxiated from non-asphyxiated term neonates when correlating with their history and clinical features.

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

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INTRODUCTION

Perinatal asphyxia is an insult to the foetus or the newborn due to lack of oxygen (hypoxia)/perfusion (ischemia) which leads to multi-organ system dysfunction including hypoxic ischemic encephalopathy and long term neuro-development disability[1,18]. Perinatal asphyxia is a common problem with the incidence varying from 0.5–2% of live births [2-4]. Around Ninety-eight percent of these neonatal deaths take place in the developing countries. Perinatal asphyxia and birth injuries together contribute to almost 29% of these deaths[5]. Many assessments tools are available to predict fetal well-being during labor and following delivery. These include electronic fetal heart rate monitoring via a cardio-tocograph, APGAR score and the assessment of fetal acid-base balance. The signs of asphyxial injury are nonspecific and overlap with other illnesses and it is difficult to diagnose perinatal asphyxia retrospectively in the absence of perinatal records[20]. Transient myocardial ischemia (TMI) with myocardial dysfunction occurs in 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 [6, 19]. Leakage of intracellular enzymes such as Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Lactate dehydrogenase (LDH) signalling multi organ dysfunction is seen together with HIE after perinatal asphyxia[7]. We conducted this study to ascertain whether CK-MB and LDH enzyme assays can distinguish an asphyxiated from a non-asphyxiated neonate.

AIMS & OBJECTIVES: 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.

To differentiate HIE neonates from non-HIE ones on the basis of significant rise of LDH & CK-MB.

MATERIALS AND METHODS: This is a Prospective cross-sectional analytical study conducted on asphyxiated and non-asphyxiated term neonates recruited from Neonatal Intensive Care Unit (NICU) and neonatal wards Nepalgunj Medical College, Nepalgunj, Nepal from January 2019 to November

2019. Cases and Controls comprised of asphyxiated and non-asphyxiated babies, respectively.

Sample size was calculated based on expected frequency of HIE in newborns with 5 min Apgar score < 6. Since Newborns with 5 minute Apgar score < 6 was found to be a risk factor for HIE, based on several studies in literatures, where it was found that 30% of newborns with 5 minute Apgar score < 6 had HIE. To detect an ODD's Ratio of 3.68 with 80% power & 95% confidence interval &1:1 case to control ratio the final sample size using EPI-INFO STATCAL software (version 6) was estimated at 75 cases & 75 controls. Adding 10 % non-response rate, the final sample size was equal to 82 cases & 82 controls.

Case group: Included 82 neonates fulfilling the following 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 [21].

Exclusion criteria 1) Congenital malformations. 2) Maternal drug addiction. 3) Neonates born to mothers who would had received magnesium sulphate within 4 hours prior to delivery or opiods (pharmacological depression) & 4) Hemolytic disease of the newborn.

Control group: Included 82 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. Levene MI staging was used to grade the severity of HIE. The cases were also observed for other systemic effects of asphyxia. Cord blood samples (2ml) from both cases and control groups was drawn for CK-MB & LDH estimation.

A serum CK-MB value >92.6 U/L and LDH value >580 U/L was taken as the cut-off level[9]. The case group also had other investigations and imaging studies done as required for post-resuscitation management of asphyxiated neonates and as per our NICU protocol.

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.

RESULT

The cord blood samples from 82 neonates comprising the cases and 82 neonates comprising the controls constituted the materials for the study. There were there 56 (68.3%) males and 26 (31.7%) females in case group there and 50 (60.9%) males and 32 (39.1%) females in control group. Forty-nine(59.7%) neonates weighed between 2500-3000g, 26(31.7%) between 3001-3499 g and 7(8.6%) more than 3500 g in the case group and 45(54.8%) between 2500-3000 kg, 33(40.2%) between 3001-3499 g and 4(5%) more than 3500 g in control group. The mean birth weight of neonates in our present study was 2860 ± 329 grams & 2940 ± 294 grams for cases and control group respectively which was statistically similar in both groups.

Fifty-six (68.3%) neonates were delivered by spontaneous vaginal delivery and 26 (37.1%) delivered by caesarean section in the case group. Amongst the control group, 50 (60.9%) neonates were born through spontaneous vaginal delivery and 32(39.1%) through caesarean section. All these comparisons were statistically not significant. (p value <0.05).

The mean LDH was 992.32 ± 437.25 U/L incase group and 350.17 ± 104.89 U/L in control group neonates. The mean value was statistically significant in neonates from case group as compared to neonates of controls group with P value <0.001. The mean CK-MB level was 71.082 ± 50.16 U/L in the case group and 44.049 ± 13.79 U/L in thecontrol group respectively. The mean value was statistically significant in neonates from case group as compared to neonates of control group with P value <0.001 as shown in Table I.

LDH	Cases	Controls	P value
Mean	992.32 ± 437.25	350.17 ± 104.89	P < 0.001
Median (25th–75th centile)	626.50 - 1262	299.25 – 413.75	
CKMB			P < 0.001
Mean	71.082 ± 50.16	44.049 ± 13.79	
Median (25th–75th centile)	63 (34.75-85)	41 (32-55)	

Table I: Comparison of mean values of LDH and CK-MB in cases and controls

The number of neonates having CK-MB levels of >92.6 U/L was statistically very significant in neonates from case group when compared to neonates of control group with P value <0.001 as shown in Table II.

CKMB cut-off	Cases	Controls	Total	P value
<92.6 U/L	69 (45.7%)	82 (54.3%)	151 (100%)	<0.001
>92.6 U/L	13 (100%)	0 (0%)	13(100%)	
Total	**82 (50%)**	**82 (50%)**	**164 (100%)**	

Table II: Comparison of cut-off levels of CK-MB cases and controls

Ninety-two (56.1%) neonates had LDH levels of <580 U/L out of which 11(12%) neonates were from case group and 81(88%) neonates were from control group. Remaining 72(43.9%) neonates were found to have LDH levels >580 U/L out of which 71(98.6%) neonates were in case group and 1(1.4%) neonates were in control group. The number of neonates having LDH levels of >580 U/L was statistically very significant in neonates from case group when compared to neonates of controls group with P value < 0.001 as shown in Table III.

LDH Cut-off	Cases	Controls	Total	P value
<580 U/L	11 (12%)	81 (88%)	92 (100 %)	<0.001
>580 U/L	71 (98.6%)	1 (1.4%)	72 (100%)	
Total	**82 (50%)**	**82 (50%)**	**164**	

Table III: Comparison of cut-off levels of LDH cases and controls

The cut-off CK-MB value of >92.6U/L had sensitivity of 18.06%, specificity of 100%, positive predictive value of 100% and negative predictive value of 60.93%. The cut-off LDH value of >580 U/L had sensitivity of 91.67%, specificity of 93.48%,positive predictive value of 91.67 % and negative predictive value of 93.48% in the newborns studied. The diagnostic performance of LDH seemed to be better than CK-MB in the newborns with perinatal asphyxia as shown in Table IV.

Cut-off	Sensitivity	Specificity	PPV	NPV	
CKMB	>92.6	18.06%	100%	100%	60.93%
LDH	>580	91.67%	93.48%	91.67%	93.48%

Table IV: Sensitivity, specificity and predictive values of CK-MB and LDH.

The ROC curve of LDH and CKMB shows that LDH is having more diagnostic value than CKMB in neonates with perinatal asphyxia with more Area under ROC (Receiving operating Characteristic) value when compared to CK-MB (0.978vs. 0.731).

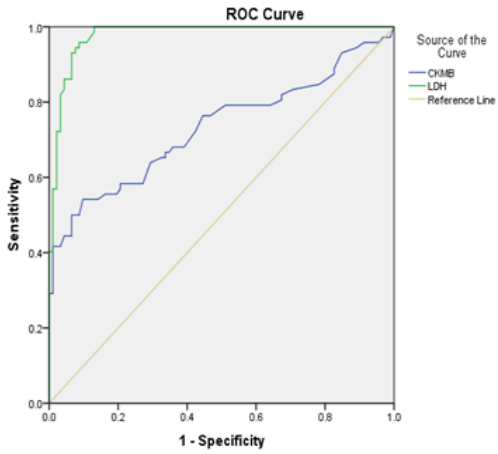

Figure 1: Shows comparison of receiver operator characteristics (ROC) curves of LDH & CK-MB

DISCUSSION

This case control study was conducted in Department of Pediatrics & Adolescent Medicine, NGMC, Nepalgunj, Nepal. Perinatal asphyxia is one of the major causes of neonatal death in developing countries and accounts for an estimated 23% of annual 4 million neonatal deaths[5]. Hypoxic ischaemic insult is seen in various organs of the body with release of several enzymes such as LDH, AST, ALT, CK-MB and Troponins. Lactate dehydrogenase is a non-specific enzyme released from various tissues – like heart, RBCs & WBCs, kidneys, lungs, liver, skeletal muscles, lymph nodes etc and CK-MB is mainly a cardiac enzyme but it is also released in small amounts from skeletal muscle injury. Significant elevation in the levels of CK-MB and LDH can serve as markers of asphyxia. In asphyxiated neonates, hypoxia is often responsible for myocardial ischemia and leads to myocardial damage.

Several studies have been conducted to evaluate better markers that help to differentiate asphyxial and non-asphyxial etiology in neonates. Primhaket al[10] 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[11] 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[12] found that antepartum fetal distress is associated with release of CK-BB, and CK-MB; therefore, these biochemical markers indicate either brain or myocardial damage. Barberi et al[13] 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[14] 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[15]. Rajakumar PS et al[16] observed that the cardiac enzymes, cTnT and CK-MB, were significantly elevated in cases when compared with controls. In 2010, Karlsson M et al[17] 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%.

Our study showed that amongst the 164 neonates studied, 92(56.1%) neonates had LDH levels of <580 U/L out of which 11(12%) neonates were from case group and 81(88%) neonates were from control group. Remaining 72(43.9%) neonates were found to have LDH levels >580 U/L out of which 71(98.6%) neonates were from case group and one (1.4%) neonate was from control group. The number of neonates having LDH levels of >580 U/L was statistically very significant in neonates from case group when compared to neonates of controls group with P value < 0.001. This is similar to the study by Reddy S et al[15] in which 100% of cases had LDH levels >580U/L. In our study the mean LDH levels in neonates from cases group (992.32 ± 437.25 U/L) were significantly higher as compared to neonates from controls group (350.17 ± 104.89 U/L) with P<0.001 which is comparable to the results of study by Reddy S et al. In the present study, the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of LDH were 91.67%, 93.48%, 91.67%, and 93.48% respectively. This is comparable to Reddy S et al in which sensitivity, specificity, PPV and NPV were 100%, 89%, 92% and 100% respectively. In study by Rajakumar PS et al[16] sensitivity and specificity of LDH were 56.5% and 75.7 % respectively which is similar to our study.

	Sensitivity	Specificity	PPV	NPV
Reddy S et al	100%	89%	92%	100%
Karlsson et al	100 %	97%	87%	100%
Present study	91.67%	93.48%	91.67%	93.48%

Comparative studies of diagnostic performance of LDH

The area under the ROC curve for LDH is 0.978 (excellent test) in our present study which is comparable to 0.998 (excellent test) in study of Reddy S et al[15] In a study conducted by Barberi, et al[13] reported that CK, CK-MB, CK-MB/CK ratio and LDH were all increased in the asphyxiated group, while in a group of neonates with respiratory distress; only CK-MB and the CK-MB/CK ratio were abnormal. Lackmann, et al[7] found that newborn infants with asphyxia have significantly higher values of SGOT, LDH & hydroxyl butyrate compared to neonates with only respiratory distress syndrome (RDS), and presence of RDS among asphyxiated neonates did not alter the enzyme levels. Karlsson M et al[17] in their clinical and experimental study done in 2008 on evaluation of organ damage in perinatal asphyxia concluded that in asphyxiated infants with different 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%. Present study shows that estimation of CK-MB at 8 hours of life and LDH at 72 hours of life could help to distinguish an asphyxiated from a non-asphyxiated term neonate with reasonable degree of accuracy. LDH was having more diagnostic value than CK-MB with more Area under ROC curve value when compared to CKMB (0.978 vs. 0.731) in our study.

CONCLUSION & RECOMMENDATIONS

LDH levels of >580 U/L and CK-MB levels of >92.6 U/L were statistically significant in neonates from case group when compared to neonates of controls group. LDH is having more diagnostic value than CK-MB in neonates with perinatal asphyxia with more Area under the ROC (Receiving operating Characteristic) value when compared to CK-MB (0.978vs. 0.731) which helps to differentiate asphyxiated from non-asphyxiated neonates. In resource poor settings these bedside diagnostic tests are having high specificity and sensitivity with low cost and good feasibility. Hence, these markers (LDH and CK-MB) can be very useful to differentiate HIE newborns from non-HIE newborns which will help in appropriate management and better outcome of these newborns.

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