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Original Research Article

Correlation of serum creatinine kinase muscle-brain fraction and lactate dehydrogenase with severity of hypoxic ischemic encephalopathy in perinatal asphyxia in term neonates

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ABSTRACT

Background: Birth asphyxia in neonates significantly contributes to their mortality and morbidity, as it leads to hypoxic ischemic encephalopathy (HIE) and multi organ dysfunction. The present study was conducted with an objective to ascertain whether serum levels of creatinine kinase muscle-brain fraction (CK-MB) and lactate dehydrogenase (LDH) can distinguish an asphyxiated from a non-asphyxiated term neonate and correlation of these enzymes cut-off levels with severity of HIE in asphyxiated term neonates.
Methods: This prospective study was conducted at Sree Mookambika Institute of Medical Sciences, Kulasekharam, Kanyakumari from September 2012 to December 2013. The study included 50 cases (asphyxiated neonates) and 50 controls (non-asphyxiated neonates) after fulfilling the requirements of inclusion criteria. Their blood samples were collected at 8±2 hours and 72±2 hours of age for estimation of CK-MB and LDH respectively and sent for analysis. The values were compared between cases and controls by using descriptive statistics.
Results: The mean CK-MB level at 8±2 hours and mean LDH level at 72±2 hours were significantly higher in cases compared to controls with $p<0.001$. Among the 50 neonates in case group, 19 (38%) had clinical evidence of HIE. Of them 3 (6%) had mild HIE, 12 (24%) had moderate HIE and 4 (8%) had severe HIE during the course in NICU. The correlation of Apgar scores of 0-3 and 4-6 at 1 minute and 5 minutes with the severity of HIE and the correlation of cut-off CK-MB level of 92.6 U/L and cut-off LDH level of 580 U/L with the severity of HIE were not significant ($p>0.05$).
Conclusions: We conclude that estimating the levels CK-MB at 8 hours of life and LDH at 72 hours of life can help to distinguish an asphyxiated from a non-asphyxiated term neonate with reasonable degree of accuracy but the enzyme cut-off levels do not correlate with severity of HIE in asphyxiated term neonates.

Keywords: Hypoxic ischemic encephalopathy, Lactate dehydrogenase, Perinatal hypoxia, Serum creatinine kinase muscle brain fraction

INTRODUCTION

Birth asphyxia is a medical condition that occurs due to impairment of blood gas exchange during birth resulting in lack of oxygen and accumulation of carbon dioxide in the body cells leading to ischemia. This results in neuronal cell death and brain damage. Asphyxia also leads to damage of vital organs such as kidney, heart and liver.[1,2]

World Health Organization (WHO), estimates that annually 4 million deaths occur due to birth asphyxia, representing 38% of all deaths of children under 5 years of age.[1] Data from National Neonatal Perinatal database (NNPD) suggests that perinatal asphyxia contributes to almost 20% of neonatal deaths in India. In India, 8.4% of newborn babies have a 1 minute Apgar score <7 and 1.4% suffer from hypoxic ischemic encephalopathy (HIE).[3]

Perinatal hypoxia can be identified by using variety of markers including electronic fetal heart monitoring, low Apgar scores, cord pH, electroencephalograms (EEG), computed tomography (CT) and magnetic resonance imaging (MRI) scans and Doppler flow studies.[4] But still there is a lack of precise identifying marker for distinguishing an asphyxiated from non-asphyxiated neonates.

Perinatal asphyxia may result in multiorgan dysfunction leading to fatal complications. Transient myocardial ischemia (TMI) with myocardial dysfunction may occur in any neonate with a history of perinatal hypoxia.

An elevated serum creatinine kinase muscle-brain fraction (CK-MB) fraction or cardiac troponin T (cTnT) level may be helpful in determining the incidence of myocardial damage. An elevated range of serum CK-MB fraction (>5% to 10%) indicates the myocardial injury.[5] Elevated serum levels of liver enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) also helps in recognizing the extent of brain damage, as liver cell injury was also seen together with HIE during perinatal asphyxia.[6-8]

The study was conducted to compare the serum levels of CK-MB and LDH among asphyxiated and non-asphyxiated term neonates and to ascertain whether these enzymes can distinguish an asphyxiated from a non-asphyxiated term neonate and correlation of these enzymes levels with severity of HIE in asphyxiated term neonates.

METHODS

This prospective study was conducted on 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 (n=50) and controls (n=50) constituted of asphyxiated and non-asphyxiated term neonates respectively.

Selection criteria

Cases included neonates with gestational age ≥37 weeks and with atleast 3 of the following signs of asphyxia:

A) Intrapartum signs of fetal distress, as indicated by non-reassuring NST on continuous electronic fetal monitoring and/ or by thick meconium staining of the amniotic fluid. B) Apgar score of <7 at one minute of life. C) Resuscitation with >1 minute of positive pressure ventilation before stable spontaneous respiration. 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.[9]

Neonates with congenital malformations, maternal drug addiction, neonates born to mothers who had received magnesium sulphate within 4 hours prior to delivery or opiods (pharmacological depression), hemolytic disease of the newborn were excluded from the study.

Controls included healthy term 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.

After getting approval from institutional ethics committee, subjects were enrolled after obtaining informed consents from their parents. On a precoded proforma, a detailed maternal history, fetal assessment details (NST), birth details, meconium stained amniotic fluid, Apgar scores at 1 and 5 minutes, birth weight and sex of the newborn were documented.

Assessment of gestational age was done based on last menstrual period and New Ballard scoring. If umbilical arterial blood sample was obtained, arterial blood gas analysis (ABG) was done to document acidosis.

The asphyxiated neonates (case group) were admitted in NICU and post-resuscitation management done. The neonates were monitored for features of HIE and other complications of asphyxia. The severity of HIE was graded using clinical grading system by Levene MI.[9]

Table 1: Hypoxic-Ischaemic Encephalopathy (HIE) clinical grading system by Levene MI.

Feature	Mild	Moderate	Severe
Consciousness	Irritable	Lethargy	Comatose
Tone	Hypotonia	Marked hypotonia	Severe hypotonia
Seizures	No	Yes	Prolonged
Sucking/respiration	Poor suck	Unable to suck	Unable to sustain Spontaneous respiration

CK-MB and LDH enzyme level estimation was done for all the neonates included in the study (the case and control group). Blood samples for CK-MB level was obtained at 8±2 hours and for LDH level was obtained at 72±2 hours of age.[10-11] Serum CK-MB level was estimated by Immuno-inhibition method and serum LDH level estimated by German Society for Clinical Chemistry (DGKC) method. The normal reference range for upper limit of CK-MB at 5-8 hours of age is 7.9% of 1,175 U/L which is ~92.6 U/L.[12] A serum CK-MB level >92.6 U/L at 8 hours was therefore taken as the cut-off level. The normal reference range of LDH in neonates and infants <1 year is 170-580 U/L.[12] A serum LDH level >580 U/L at 72 hours of age was therefore taken as the cut-off level.

Other necessary investigations as needed in the management of an asphyxiated neonate were done. Relevant investigations in evaluation of cause for seizures other than HIE were done. Sepsis screen and peripheral smear for hemolysis were done in neonates of case group.

Statistical analysis

Descriptive statistical analysis was carried out in the present study. Results on continuous measurements are presented on Mean±SD (min-max) and results on categorical measurements are presented in number (%). Significance was assessed at 5% level of significance. Student t test (two tailed, independent) was used to find the significance of study parameters on continuous scale between two groups (inter group analysis) on metric parameters, Chi-square/Fisher exact test was used to find the significance of study parameters on categorical scale between two or more groups. Roc curve analysis was performed to find the diagnostic performance of CK-MB and LDH. The Statistical software namely SAS 9.2, SPSS 15.0, Stata 10.1, MedCalc 9.0.1, Systat 12.0 and R environment ver.2.11.1 were used for the analysis of the data and Microsoft word and Excel was used to generate graphs, tables etc.

RESULTS

Table 2 shows the demographic and clinical characteristics of both cases (n=50) and controls (n=50). The distribution of male sex, mean birth weight and primi para mothers were similar in both the groups. Non reassuring NST was documented in 39 (78%) neonates and meconium stained amniotic fluid was noted in 32 (64%) neonates in the case group. Cesarean section and instrumental delivery rates were significantly higher in the case group (88%) when compared to the control group (34%) (P<0.001). Among the neonates in case group, 50 (100%) of them had an Apgar score <7 at 1 minute and 8 (16%) of them had an Apgar score <7 even at 5 minutes of life. Abnormal neurological examination (tone abnormality) was noted in 19 (38%) cases.

Table 2: Demographic and clinical characteristics in cases and controls.

Characteristics	Cases (n=50)	Controls (n=50)	P value
Gender			
Male	30 (60)	29 (58)	0.839
Female	20 (40)	21 (42)	
Birth weight (in kgs)			
2.5-3	33 (66)	29 (58)	0.135
3-3.5	14 (28)	19 (38)	
>3.5	3 (6)	2 (4)	
Maternal history			
Primi	33 (66)	29 (58)	0.130
Multi	17 (34)	21 (42)	
Mode of delivery			
Normal	6 (12)	33 (66)	0.001
Instrumental	7 (14)	1 (2)	
Cesarean section	37 (74)	16 (32)	
Non stress test (NST)			
Reassuring	11 (22)	50 (100)	0.001
Non-reassuring	39 (78)	0 (0)	
Meconium stained amniotic fluid (MSAF)			
Present	32 (64)	0 (0)	0.001
Absent	18 (36)	50 (100)	
Apgar score at 1 min			
0-3	45 (90)	0 (0)	0.001
4-6	5 (10)	0 (0)	
≥7.0	0 (0)	50 (100)	
Apgar score at 5 min			
0-3	0 (0)	0 (0)	0.001
4-6	8 (16)	0 (0)	
≥7.0	42 (84)	50 (100)	
Neurological examination			
Tone (T)-Normal (N)	31 (62)	50 (100)	0.001
Tone-Decreased (↓) (mild and marked)	15 (30)	0 (0)	
Tone-Flaccid (F)	4 (8)	0 (0)	

Table 3: Complications observed in cases (n=50).

Complications	Number of neonates (n=50)	Percentage
HIE	19	38.0
Mild	3	6.0
Moderate	12	24.0
Severe	4	8.0
Respiratory distress	38	76
Inotrope support	6	12.0
Congestive cardiac failure (CCF)	1	2.0
Acute renal failure (ARF)	1	2.0
Death	5	10.0

As shown in Table 3, among the 50 neonates in case group, 19 (38%) had clinical evidence of HIE, 38 (76%) had respiratory distress, 6 (12%) had shock requiring inotrope support, 1 (2%) had congestive cardiac failure and 1 (2%) had acute renal failure. 5 (10%) neonates died. Of the 5 neonates which died, 3 had severe HIE and 2 had moderate HIE. 4 of them had cardiogenic shock. 2 (4%) were discharged against medical advice.

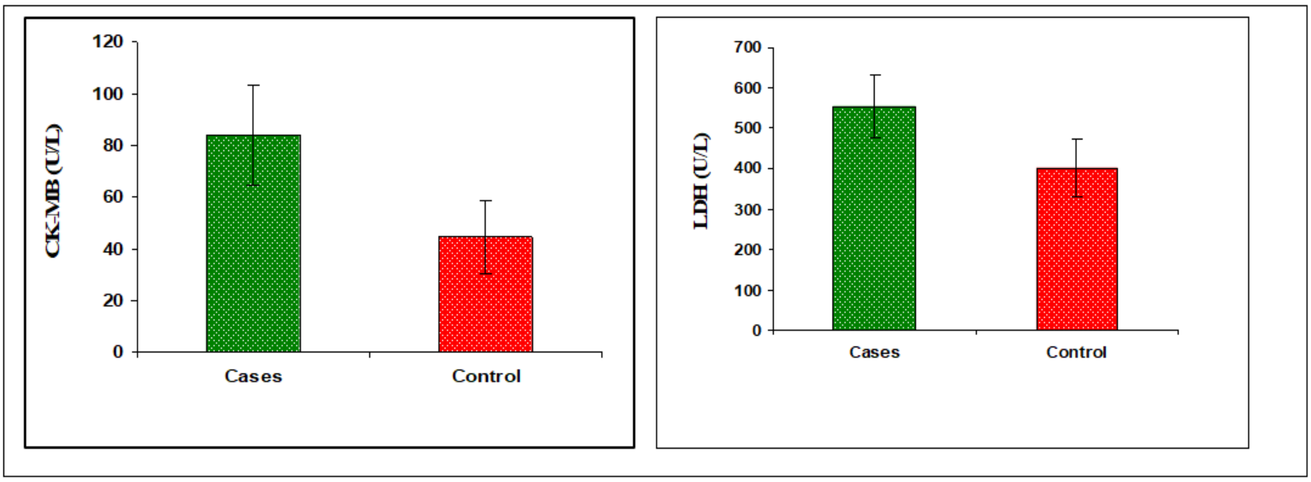

Figure 1: Mean CK-MB levels and LDH levels at 8 and 72 hours respectively among cases and controls.

Figure 1 presents the mean CK-MB levels and LDH levels at 8 and 72 hours respectively among cases and controls. The mean CK-MB level at 8±2 hours was 83.98±19.60 U/L in case group and 44.56±14.53 U/L in the control group. The mean LDH level at 72±2 hours was 555.65±105.95 U/L in case group and 402.78±85.24 U/L in the control group. The mean values were significantly higher in cases compared to controls with P<0.001.

Table 4 presents the correlation of Apgar score, CK-MB and LDH with severity of HIE in cases. Among the 50 neonates in the case group, 36 (72%) had CK-MB levels <92.6 U/L and 14 (28%) had CK-MB levels >92.6 U/L. Out of the 50 neonates, 19 (38%) developed HIE. 1 (33.3%) case of mild HIE and 3 (25%) cases of moderate HIE had CK-MB levels <92.6 U/L. None of the case with severe HIE had CK-MB levels <92.6 U/L. 2 (66.7%) cases of mild HIE, 9 (75%) cases of moderate HIE and 4 (100%) cases with severe HIE had CK-MB levels >92.6 U/L. The correlation of cut-off CK-MB level of 92.6 U/L with the severity of HIE was not significant (P=0.569).

Among the 50 neonates in the case group, 1 neonate with severe HIE died before LDH could be estimated. Out of the 49 neonates, 26 (53.06%) had LDH levels <580 U/L and 23 (46.94%) had LDH levels >580 U/L. 19 (38%) cases developed HIE. 2 (66.7%) case of mild HIE and 4 (33.3%) cases of moderate HIE had LDH levels <580 U/L. None of the case with severe HIE had LDH levels <580 U/L. 1 (33.3%) cases of mild HIE, 8 (66.7%) cases of moderate HIE and 3 (100%) cases with severe HIE had LDH levels >580 U/L. The correlation of cut-off LDH level of 580 U/L with the severity of HIE was not significant (P=0.501).

Table 4: Correlation of Apgar score, CK-MB and LDH with severity of HIE.

Variables	HIE			P value
	Mild (n=3)	Moderate (n=12)	Severe (n=4)	
Apgar score at 1 minute				
0-3	2 (66.7%)	12 (100.0%)	4 (100.0%)	0.158
4-6	1 (33.3%)	0	0	
≥7.0	0	0	0	
Apgar score at 5 minutes				
0-3	0	0	0	0.421
4-6	1 (33.3%)	4 (33.3%)	3 (75.0%)	
≥7.0	2 (66.7%)	8 (66.7%)	1 (25.0%)	
CK-MB (U/L)				
<92.6	1 (33.3%)	3 (25.0%)	0	0.569
>92.6	2 (66.7%)	9 (75.0%)	4 (100.0%)	
LDH (U/L)*				
<580.0	2 (66.7%)	4 (33.3%)	*0	0.501
>580.0	1 (33.3%)	8 (66.7%)	3 (100.0%)	

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

DISCUSSION

Facilities for estimation of CK-MB and LDH enzyme levels are routinely available in most centres and hence in the present study, an effort has been made to establish the usefulness of these enzymes in distinguishing an asphyxiated from a non-asphyxiated neonate based on previous studies and to ascertain the correlation of these enzymes cut-off levels with severity of HIE.

In the present study male preponderance was seen in both the groups and this difference in gender was similar to the observations made by Reddy et al.[13] No significant association was found between birth asphyxia and parity of mothers (p>0.05) which is comparable to Khreisat et al.[14]

Cesarean section and instrumental delivery rates were significantly higher in the case group (88%) when compared to the control group (34%) (P<0.001). The mean birth weight was similar in both the groups (p=0.135). These observations were in accordance with findings of Ahmed et al, Reddy et al and Khreisat et al.[4,10,14]

In the present study, the incidence of HIE is 38% with 6% having mild HIE, 24% moderate HIE and 8% severe HIE. The incidence is lower compared to Rajakumar et al in which 100% of the cases included had HIE.[15] The incidence in the present study is slightly higher when compared to Karunatilaka et al in which 25.71% of the cases had HIE.[16] The differences in the incidence of HIE and involvement of other organ systems in birth asphyxia in different studies could be attributable to major differences in the inclusion criteria for the cases, grading system used, limitations of non-reassuring fetal heart rate patterns, meconium-stained amniotic fluid, a low 1 min Apgar score and mild to moderate academia in predicting the extent and severity of hypoxic-ischemic injury to brain and other organs, initiation and effectiveness of resuscitative measures at birth, level of neonatal intensive care, post asphyxial monitoring and management of the asphyxiated newborns.

However the incidence of cardiogenic shock in the present study is closer to Reddy et al and Rajakumar et al.[13,15] In the present study, of the 5 asphyxiated neonates who had died, 4 of them had cardiogenic shock. This is comparable to Rajakumar et al in which all the 5 (16.7%) cases with cardiogenic shock had died.[15] The 5 deaths in the present study were attributable to either cardiac problems (CCF and shock), neurological problems (severe HIE) or renal problems (ARF).

Estimation of LDH levels at 72 hours of life of infants was the most accurate test for distinguishing asphyxiated newborns from non-asphyxiated newborns in correlation with their clinical characteristics. Elevated serum CK-MB levels helps in determining the incidence of myocardial damage in asphyxiated cases. In the present study, the mean CK-MB level at 8±2 hours (83.98±19.60 U/L) and the mean LDH level at 72±2 hours (555.65±105.95 U/L) were significantly higher in asphyxiated newborns compared to controls. Similar observations were also noticed by Primhak et al and Barberi et al.[10,17]

The correlation of Apgar scores of 0-3 and 4-6 at 1 minute and Apgar scores of 0-3, 4-6 and ≥7 at 5 minutes with the severity of HIE is not significant in this study. The correlation of cut-off CK-MB level of 92.6 U/L and cut-off LDH level of 580 U/L with the severity of HIE is not significant in the present study.

Limitations of the study are:

- One of the major limitations of the present study was the inability to document umbilical arterial pH in all the cases though the neonates had met the inclusion criteria to be enrolled as asphyxiated neonates.
- ABG analysis was done in only 6 cases. Of them, 1 neonate had pH <7 and developed severe HIE with cardiogenic shock.

CONCLUSION

The findings of the study concludes that elevated levels of serum CK-MB and LDH can help identify an asphyxiated from a non-asphyxiated term neonate and subsequent development of HIE in these asphyxiated neonates. However, the correlation of cut-off CK-MB level of 92.6 U/L and cut-off LDH level of 580 U/L with the severity of HIE were not significant (p>0.05). Hence, estimation of the these enzyme can help to identify an asphyxiated neonate who are at high risk of developing HIE and guide management of such neonates, especially in those neonates where definite history or documentation of perinatal asphyxia and resuscitation details are unavailable.

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