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CKMB and LDH Enzyme Markers in the study Of Perinatal Asphyxia

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Abstract

A systematic analysis of global, regional and national causes of child mortality in 2013 identified preterm birth complications and infections to be the two major causes of neonatal deaths in India The review, which included the data from the Million Death Study from India, found perinatal asphyxia and malformations to be the other two significant causes of neonatal mortality.

Intrapartum-related conditions or perinatal asphyxia not only leads to neonatal deaths, but also accounts for a significant proportion of stillbirths. It is difficult to estimate the true burden of asphyxia because of the different definitions used in the studies. The reported incidence varies from 2 to 16.2% in community-based studies, with the reported case fatality rates ranging from 38.5 to 74%. About 2.8 and 5.6% of all live births had moderate and severe asphyxia, respectively, in a large hospital-based study; the case fatality rate was relatively low at ~8.7%.

These findings are very similar to the overall global pattern.

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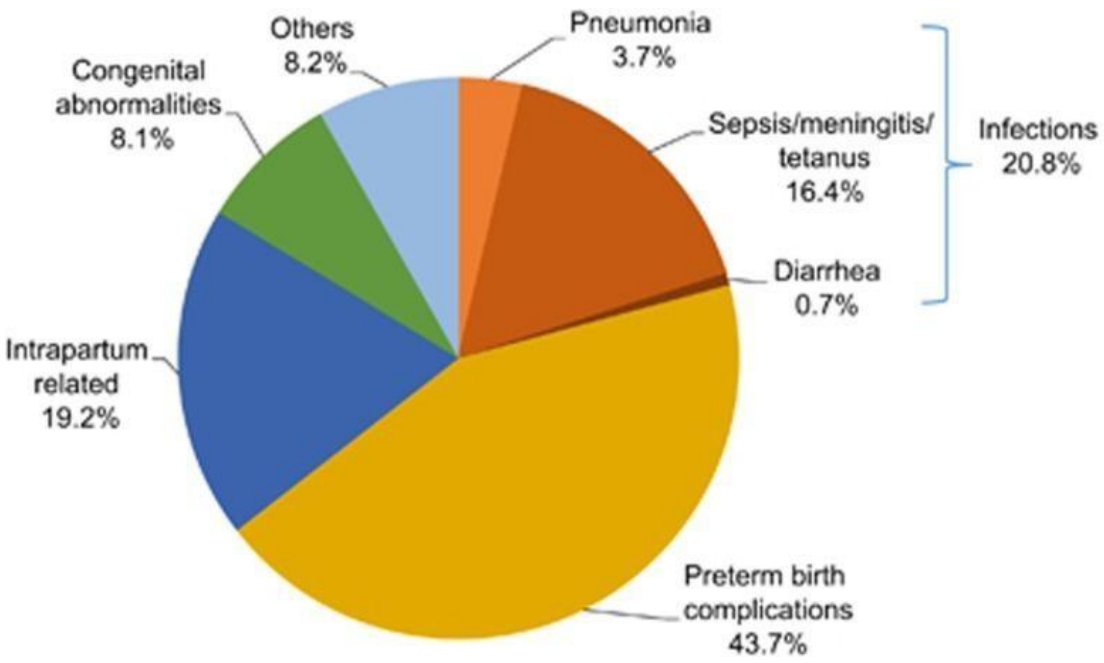

Study concluded that the country has to increase the coverage of key interventions and also improve the quality of care in health facilities on an urgent basis.

Birth Asphyxia definition[3] (WHO) –

1. Extramural babies-

- Moderate birth asphyxia-slow, gasping breathing at 1 minute of age.
- Severe birth asphyxia:No breathing at 1 minute of age.

2. Intramural babies

- Birth asphyxia: APGAR score less than 7 at 1 minute of age
- Moderate birth asphyxia: APGAR score between 4 to 6 at 1 minute ofage.
- Severe birth asphyxia: APGAR score of 3 or less at 1 minute of age.

Hypoxic-ischemic encephalopathy (HIE) due to perinatal asphyxia remains an important cause of neonatal morbidity and mortality. The neonatal symptoms and signs of mild hypoxic ischemic encephalopathy are more subtle making an early precise diagnosis more difficult. Early prediction of hypoxic ischemic encephalopathy is needed for selection of newborn infants who could benefit from neuroprotective treatment like hypothermia. In most of the cases, perinatal asphyxia is diagnosed retrospectively. However, it is difficult to diagnose in the absence of perinatal records in our country. It has been seen that neonatal mortality

correlates more strongly with the 5 min APGAR score. However, the main limitation of APGAR score is its affection by certain variables such as gestational age, maternal medications, resuscitation, cardiopulmonary, and neurological conditions and has not been found usefulto predict outcome[5].

Previous studies revealed that elevated hepatic enzymes were correlated with the degree of hypoxia[6]. Enzyme leakage as a result of hypoxia-ischaemia induced cell damage in affected organs is seen together with hypoxic ischaemic encephalopathy (HIE) after perinatal asphyxia. In hypoxic ischemic encephalopathy the injured cells leak intra-cellular enzymes, some of which are easy to measure in plasma e.g. lactate dehydrogenase (LDH), the increased level of this enzyme has been reported after neonatal asphyxia,and hence this enzyme can be used as a predictor of neonatal hypoxic

ischemic encephalopathy as it rises after cell damage following asphyxia and also can be used to detect the severity of hypoxic ischemic encephalopathy insult in the period.
Lactate Dehydrogenase (LDH) increases early in newborns in several critical conditions, and the LDH activity correlates well with the severity of diseases such as asphyxia, respiratory distress and Necrotizing Enterocolitis (NEC). Early identification and treatment can help to properly manage and prevent adverse outcome and improve long term prognosis.

Cardiac biomarkers show good correlation with echo-derived markers of myocardial function, and a significant elevation of cord blood troponin has been found to be an excellent early predictor of severity of HIE and mortality in term infants.[7]

Severe Perinatal Asphyxia has been known to cause ischemic myocardial injury with potentially fatal outcomes. 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 Serum Cardiac Troponin (cTnT) is a reliable marker of myocardial injury.

Estimation of these enzymes may help in predicting the severity of HIE and hence help in guiding timely and correct interventions.

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Aim and Objectives

AIM:

To study enzyme markers LDH and CKMB in perinatal asphyxia.

OBJECTIVES:

- *Primary objective*:
- ✓ Study the levels of enzyme markers LDH and CKMB in perinatalasphyxia.
- *Secondary objective*:

  To correlate the enzyme levels of LDH and CKMB with the
- ✓ Severity of HIE.
- ✓ Outcome of asphyxia.
- ✓ Need for intensive care procedures.

Review of Literature

Historical View

The Greek origin of the word asphyxia means without pulse. Since the sixteenth century, competition between midwives and surgeons has created a culture of blame around the difficult delivery. In the late seventeenth century, 100 years before oxygen was discovered, researchers associated ―apparent death of the newborn‖ with impaired respiratory function of the placenta. Although the semantic inaccuracy (―pulselessness‖) was debated, ―asphyxia‖ was not scientifically defined until 1992. From 1792 the diagnosis was based on a lack of oxygen.

In 1862, William Little linked birth asphyxia with cerebral palsy, and although never confirmed, his hypothesis was accepted by scientists and the public. Fetal well-being was assessed by auscultating heart beats since 1822, and continuous electronic fetal monitoring was introduced in the 1960s without scientific assessment. It neither diminished the incidence of birth asphyxia nor of cerebral palsy, but rather raised the rate of caesarean sections and litigation against obstetricians and midwives. Within each specialty studying asphyxia, once a definition was established, the exceptions were enormous. For instance, to the pathologist, an "asphyxic" lesion may occur without any clinical or biochemical history of asphyxia. The term asphyxia when defined in physiology textbooks includes hypoxia plus hypercarbia. Alternatively, biochemical evidence of asphyxia is present in tremendous numbers of children who, in fact, are clinically completely normal.

In reviewing the history of birth asphyxia, one name in perinatal medicine which should be central is that of N. J. Eastman. Dr. Eastman defined asphyxia as "an inability of the child to breathe and apnea associated with oxygen deficiency during labor." It was this very issue, the initiation of respiration at birth that stimulated Dr. Eastman's original contributions. Dr. Eastman was vitally interested in the concept of whether hypercarbia or hypoxia was responsible for the initiation of respiration. He felt that only by understanding the normal initiation of human respiration and the biochemistry involved at the time of initiation of respiration, could we know the abnormalities associated with abnormal respiration, i.e. asphyxia. His studies proceeded in aseries of five articles published between 1931 and 1936. He first studied the oxygen concentration and oxygen delivery of maternal and fetal blood through the umbilical vein and the return of blood to the mother via the umbilical artery, in 16 patients. He showed the maternal-fetal lactate relationships and indicatedthat this was likely a measure of mild oxygen deficiency. He stated that the absence of hyperlactatemia demonstrated fetal oxygen adequacy. He quoted apaper by Heinbicken, a German investigator, who in 1929 demonstrated that cellular acidic products generated from anoxemia could cause cellular damage. The summary of these two inquiries and their clinical application is encompassed by Eastman's third paper on the subject. He quoted the Kane and Kreiselman study of 1930 showing increased carbon dioxide in the blood of asphyxiated adult patients. Dr. Eastman then measured the carbon dioxide and pH in normal and abnormal fetal and maternal blood. Lastly, he demonstrated that neonatal acidosis accompanies asphyxia.

In a study by **Shylaja et al**[21] in 2014,on 50 asphyxiated neonates comprisingthe cases and 50 non-asphyxiated neonates comprising the controls, serum CK-MB at 8 hours and 24 hours and serum LDH were performed. A serum CK-MB value >92.6U/L at 8 hours, >60 U/L at 24 hours and LDH value >580 U/L at 72 hours was taken as the cut-off level. The sensitivity, specificity, Positive predictive value (PPV), Negative predictive value (NPV) was calculated for both CK-MB and LDH. It was found that he cut-off CK-MB value of >92.6 U/L at 8 hours had 82% sensitivity with a specificity of 80%. CK-MB had a positive predictive value of 80.34% with a negative predictive value of 81.63%.The cut-off CK-MB value of >60 U/L at 24 hours had 58.33% sensitivity with a specificity of 95.83%. CK-MB had a positive predictive value of 93.33% with a negative predictive value of 69.70%.The cut-off LDH value of >580 U/L at 72 hours had 100% sensitivity with a specificity of 88%. LDH hada positive predictive value of 89.29% with a negative predictive value of 100%. It concluded

that estimation of CK-MB at 8 hours and 24 hours of life and LDH at 72 hours of life can help distinguish an asphyxiated from a non- asphyxiated neonate in correlation with history and clinical features in the neonate. The diagnostic performance of LDH is better than CK-MB.

In a 2014 study by **Beken S et al**[22] they investigated the predictive values of biochemical parameters, including serum creatine kinase (CK), lactate dehydrogenase (LDH), uric acid (UA), and lactate, in newborns with HIE. A total of 94 patients who were diagnosed with HIE were prospectively enrolled into the study. According to the Sarnat and Sarnat classification, 29 (30.9%) patients had Stage I, 36 (38.3%) Stage II, and 29 (30.9%) Stage III HIE. WhenCK, LDH, UA, and lactate were used together in order to determine the stage of HIE, specificity and sensitivity were calculated to be 87% and 94%, respectively. They found that measurement of serum CK, LDH, lactate, andUA levels together is a promising method in determining the stage of hypoxia in the laboratory before clinical manifestations occur so that hypothermiatreatment can be initiated earlier.

In a 2015 study by **Vargas et al**[23],they concluded that perinatal asphyxia may be diagnosed in any hospital if the neonatologist or the neurologist apply the easy clinical score of Sarnat and Sarnat, the iso-enzyme CKMB and the serial ultrasonography. In this study the worse alteration was with 72 hours of life, however they noted that they must be careful because in one neonate the alteration was present only with 28 days of life.

A 2015 review by **Rabindran et al**[24] concluded that excellent diagnostic ability of serum LDH for asphyxia has been reported in literature. Raised Serum LDH level in the first six hours predicted the development of HIE between 6- 72 hours after birth. At a cut-off value of 2812 IU/L, it had 90% sensitivity, 96.7% specificity, 96.4% PPV & 91% NPV for diagnosis of HIE . In a retrospective study, serum LDH successfully predicted an abnormal mental or psychomotor development index at 18 months of age in neonates with HIE.Levels of CK-MB began to rise within the first few hours of life and are significantly higher in moderate and severe grades of HIE compared with mild grades and normal controls within the first 2–4 hour.

In the study by **Kanimozhi et al**[36] in 2015, they found that the diagnostic performance of LDH is better than CK-MB. Estimation of CK-MB and LDH level at 8 hours and 72 hours

of life can distinguish an asphyxiated from a non asphyxiated term newborn in correlation with history and clinical features in the neonate.

In a 2016 study by **Samad et al**[25],they concluded that :There was a higher rate of alteration in platelet count, levels of LDH, AST, ALT, urea , creatinine and bilirubin in asphyxiated infants. These alterations may be correlated with damage of vital organ of asphyxiated neonates.

A 2016 study by **Patra et al**[26] included 75 asphyxiated neonates as case and 75 healthy neonates as controls. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and lactatedehydrogenase (LDH) levels were estimated by IFCC method. Data gathered from perinatal asphyxia patients and control patients in a preformed pro forma were analyzed by appropriate statistical methods. Serum AST, ALT, LDH, and ALP were found significantly higher in asphyxiated babies compared to the control group ($P < 0.05$). The rise of AST, ALT, and LDH also showed asignificant positive correlation with the severity and outcome of asphyxia. They concluded that estimation of hepatic enzymes can be used as a markerto diagnose the presence of perinatal asphyxia and also to assess its severityand outcome.

In a 2016 study by **Merchant et al**[35] they concluded that severe ECG changes (Grades 3 and 4), CK-MB elevation and reduced fractional shortening on echocardiography can be considered as reliable marker ofmyocardial ischemia in perinatal asphyxia.

In a 2016 study in neonates with perinatal asphyxia by **Saha et al**[113] they found significantly greater increase in CKMB and LDH levels with increasing severity of HIE.

In 2017, study by **Nakajima et al**[27] retrospectively studied neonates whose 1 minute APGAR score was <7. They concluded that the therapeutic hypothermia group showed significantly larger base deficit, and higher lactate, AST, ALT, LDH, and CK (all $p < 0.01$). The duration of mechanical ventilation significantly correlated with AST, ALT, LDH, and CK levels (all $p < 0.01$). Theyconcluded that initial enzyme levels are useful for predicting the duration of mechanical ventilation in stressed neonates.

In a 2017 multicenter retrospective study by **Muniraman et al**[28] they compared hepatic

biomarkers obtained during the first postnatal week, according to the severity of HIE and whether treated with TH. Of a total of 361 infants with HIE, 223 (62%) received TH and 138 (38%) were managed at normal temperature. Most hepatic biomarkers and C-reactive protein (CRP) were significantly associated with the severity of HIE ($p < 0.001$). Infants treated with TH had lower peak alanine aminotransferase (ALT) concentrations ($p = 0.025$) and a delay in reaching peak CRP concentration ($p < 0.001$).They observed a significant association between the clinical grade of HIE and biomarkers of liver metabolism and function. Therapeutic hypothermia was associated with delayed CRP responses and with lowerALT concentrations and so may have the potential to modulate hepatic injury.

In a 2017 study by **Jones et al**[29], infants qualifying for therapeutic hypothermia (TH) based on aEEG abnormalities were considered to have HIE ($n = 13$; 16.5%), whereas babies with normal aEEG were classified as ―non-HIE‖ ($n = 66$; 83.5%). The highest AUC measure was associated with the five- minute APGAR score (0.89 (0.79–0.99)). Troponin T (0.81 (0.64–0.98)) and ALT (0.78 (0.60–96)) also showed high values.They concluded that the APGAR score, troponin T and ALT were found to be strong and useful predictors of HIE.

In 2017 study by **Ashraf et al**[114]they found that cardiac and liver enzymes were deranged and were significantly related to severity of HIE.

In a study by **HM Sanjay et al**[110] published in 2018,they found that estimating CKMB at 8 hours and LDH at 72 hours of life can help to distinguish a term asphyxiated neonate from a non -asphyxiated one as they were significantly higher in the first group.

In a 2018 study by **Joseph S et al**[30] they found that in asphyxiated term neonates, early cTnT elevation is a marker for predicting myocardial dysfunction and elevated cTnT levels had high sensitivity and specificity. There was significant relation with increasing cTnT values and increasing grades of HIE.

In a 2018 study by **Graham et al**[31] they concluded that although new single biomarker studies continue to emerge, utilizing metabolic profiling to more comprehensively understand the effects of injury and treatment on entire pathways following neonatal brain injury may identify new therapeutic targets in neonatal brain injury. A number of biomarkers used to identify other conditions are now being used to identify neonatal neurologic injury.

Markers of cardiac injury are increasingly useful in this respect. Cytokine biomarkers in a relevant preclinical model of HIE identify a proinflammatory surge during the rewarming period following therapeutic hypothermia. If confirmed, these studies may reveal an additional therapeutic target in neonatal HIE. Until recently, very few biomarker studies in neonatal brain injury included post hospital outcomes. Future studies will need to include these data to provide maximal information about their utility.

A panel of multiple inflammatory and neuronal biomarkers measured via a point of care bedside tool at various time points is likely to be the most accurate way to identify and assess the severity, timing and pattern of injury. Furthermore, full pathway analysis via various strategies may identify new therapeutic targets for neonatal hypoxia-ischemia brain injury. Neonatal brain biomarker research is currently in its very early development with major advances still to be made.

A 2018 study by **K Meena et al**[32] found that estimation of CK-MB at 8 hours oflife and LDH at 72 hours of life can help distinguish an asphyxiated from a nonasphyxiated term neonate in correlation with history and clinical features in the neonate.

A 2018 study by **Valinjkar et al**[33] found clear relationship between clinical pattern of asphyxiated newborns and alterations of enzymatic and electrocardiographic parameters. Neonates with severe hypoxic damage reflected significant changes in ECG and enzyme levels. It was concluded that unlike CK-MB, Serum Cardiac Troponin-T concentrations are significantly higher in asphyxiated neonates who develop cardiac dysfunction.

A study by **Paliwal et al**[34] in 2018 found that mean serum values of CK-MB were found to be decreased on day 3 in asphyxiated neonates and a negative correlation was seen between day 1 and 3 for CK-MB. The mean values of CK-MB were decreased in different stages of HIE on day 3 as compared today 1 and a negative correlation was observed between day 1 and day 3 for CK-MB in no HIE, HIE I, HIE II and HIE III stages. They concluded that serum CK-MB concentrations were increased considerably after birth asphyxia, and the increase is associated with the severity of HIE with a poorer outcome.

In a study by **Chawla et al**[37] in 2019 they found that the cut-off LDH value of >580 U/L had 91.67% sensitivity with a specificity of 93.48%. LDH had a positive predictive value of

91.67 % with a negative predictive value of 93.48% in the newborns studied. The cut-off CK-MB value of >92.6U/L had sensitivity of 18.06% with a specificity of 100%. CK-MB had a positive predictive value of 100% with a negative predictive value of 60.93% in newborns studied. They concluded that LDH is having more diagnostic value than CK-MB in neonates with perinatal asphyxia which helps to differentiate asphyxiated from non-asphyxiated neonates. In resource poor settings these markers can be very useful to differentiate HIE newborns from non-HIE newborns.

Stress vs. distress

It is clear from the above that the fetus is a beautifully adapted organism witha number of interrelated mechanisms to protect it from the rigors of labor,both hypoxic and ischemic. Stress is an invariable accompaniment of the birth process and one which the fetus is well able to withstand under most circumstances. Distress may result from a prolonged stress response and the two may merge as an imperceptible continuum. It may be extremely difficult to separate fetal stress from distress using currently available clinical methods. Fetal distress may occur as the result of a single period of hypoxia which is too long, or periods which occur too frequently. Currently, methods used to detect 'fetal distress' such as CTG and fetal scalp pH assessments are really detecting degrees of fetal stress. These tests may be misinterpreted by the obstetrician as fetal distress, but an understanding of the fetal responsesto the stress of uncompromised labor might encourage the fetus' medicalattendants that she/he requires no assistance [56].

Multiorgan dysfunction (MOD)

The fetus copes with an asphyxial event by a number of protective reflexesto preserve function to vital organs. Less well-perfused tissues may be particularly vulnerable to hypoxic-ischemic injury. In a term infant with perinatal asphyxia renal, neurologic, cardiac and lung dysfunction occurs in 50%, 28%, 25% and 23% cases respectively. The kidney appears to be most vulnerable, followed by the brain and then the heart. Gastrointestinal complications of asphyxia are uncommon (Table:7).

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Table 1: Multiorgan Systemic Effects of Asphyxia. [69]

SYSTEM	EFFECT
Central nervous system	Hypoxic-ischemic encephalopathy, infarction, intracranial hemorrhage, seizures, cerebral edema, hypotonia, hypertonia
Cardiovascular	Myocardial ischemia, poor contractility, cardiac stun, tricuspid insufficiency, hypotension
Pulmonary	Pulmonary hypertension, pulmonary hemorrhage, respiratory distress syndrome
Renal	Acute tubular or cortical necrosis
Adrenal	Adrenal hemorrhage
Gastrointestinal	Perforation, ulceration with hemorrhage, necrosis
Metabolic	Inappropriate secretion of antidiuretic hormone, hyponatremia, hypoglycemia, hypocalcemia, myoglobinuria
Integument	Subcutaneous fat necrosis
Hematology	Disseminated intravascular coagulation

Lungs

During intrapartum asphyxia, the fetus commonly passes meconium and gasping may occur due to brainstem compromise. The gasp causes meconium to be aspirated deep into the bronchial tree and this may cause a chemical pneumonitis with severe pulmonary hypertension and a high risk of air leak. Those infants who are pharmacologically paralyzed in order to facilitate mechanical ventilation for meconium aspiration syndrome will not show clinical signs of encephalopathy and coincidental cerebral injury may not be recognized. These infants should have continuous EEG monitoring to assess cerebral function [56].

Cardiovascular system

Blood flow to the myocardium is preserved during asphyxial episodes but cardiac compromise is a relatively common complication of hypoxic- ischemic injury. Myocardial

dysfunction detected by Doppler ultrasound studies has been reported in 28-40% of asphyxiated infants[70]. Recognized complications include cardiogenic shock and hypotension, functional tricuspid incompetence secondary to acute cardiac dilation, arrhythmias and myocardial ischemia which may be diagnosed from the electrocardiogram. The electrocardiography may show ST depression in the midprecordium and T- wave inversion in the left precordium. Echocardiographic findings include decreased left ventricular contractility, especially of posterior wall; elevated ventricular end-diastolic pressures; tricuspid insufficiency and pulmonary hypertension due to poor ventricular function. In severely asphyxiated infants, dysfunction more commonly affects the right ventricle. A fixed HR mayraise suspicion of clinical brain death [41].

Renal impairment

The kidney is the most common organ to be affected in perinatal asphyxiaThe proximal tubule of the kidney is especially affected by decreased perfusion. Acute tubular necrosis and oliguria occurs commonly following episodes of asphyxia. This usually recovers with supportive treatment alone. The incidence of renal impairment (oliguria) after birth asphyxia occurs in 23- 55% of babies and acute renal failure was reported in 19% of asphyxiated infants [72]. Acute retention of urine is also a relatively common complication following birth asphyxia and usually indicates very severe compromise, often associated with severe cerebral injury. Renal failure following asphyxia has also been reported to be due to myoglobinuria [56]

Gastrointestinal tract

Necrotizing enterocolitis is associated with hypoxic-ischemic events but in mature infants this is rarely seen in conjunction with HIE [56].

Metabolic disorders

One of the commonest metabolic complications of birth asphyxia is inappropriate antidiuretic hormone secretion with concentrated urine, dilute plasma and hyponatremia. Transient hyperammonemia has been reported with asphyxia but the precise cause of this metabolic compromise is not known [56].

Hematological disorders

Disseminated intravascular coagulation (DIC) is a well- recognized complication of birth asphyxia and usually presents with excessive bleeding from puncture sites together with

petechial hemorrhages. Secondary complications such as intracranial hemorrhages may occur as the result of theDIC [56]. The combined criteria for MOD are based on biochemical and clinical measurements and differ somewhat between different studies [71, 73, 74]. Criteria used by Shah et al [73]are listed in Table:8.

Table 2: Criteria for organ dysfunction in newborn infants with perinatal asphyxia used by Shah et al 2004. [73]

Renal: anuria or oliguria (< 1ml/kg/h) for 24h or more, and a serum creatinine concentration > 100mmol/L; or anuria/oliguria for > 36h; or any serum creatinine > 125mmol/L; or serial serum creatinine values that increase postnatally
Cardiovascular: hypotension demanding treatment with an inotrope drug for more than 24h to maintain blood pressure within the normal range, or electrocardiographic evidence of transient myocardial ischemia
Pulmonary: need for ventilator support with oxygen requirement > 40% for at least the first four hours after birth
Hepatic: AST > 100U/L or ALT > 100U/L at any time during the first week after birth

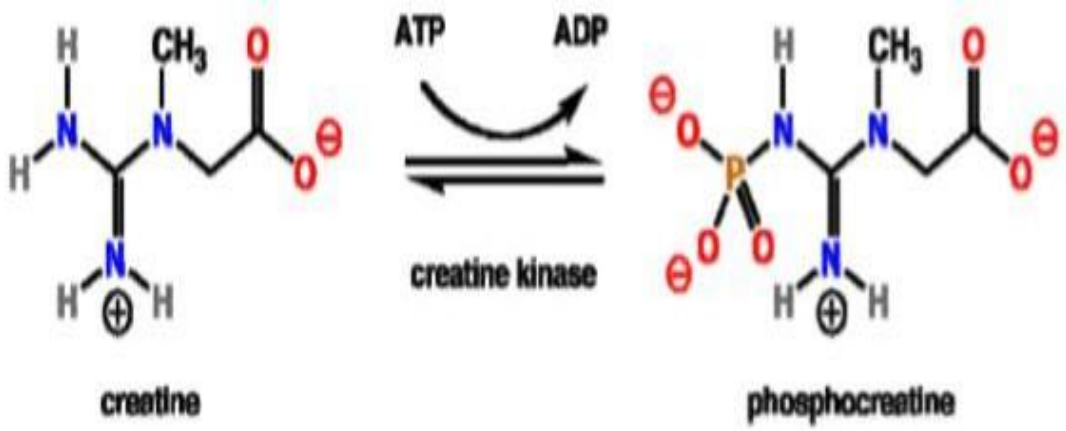
The gamut of organ involvement in perinatal asphyxia varies among series, depending in part upon the definitions used for asphyxia and organ dysfunction. In a retrospective study of 130 term infants with asphyxia, the proportion of those with organ dysfunction was: renal 70%, cardiovascular 62%, pulmonary 86%, hepatic 85% [73] These proportions are similar across different studies [73, 74.] In another series of 152 asphyxiated term infants followed prospectively, neurologic and systemic complications occurred in 43% and 57%, respectively. Organ dysfunction included respiratory abnormalities 39%, infection 17%, gastrointestinal intolerance 15%. Infants were considered to have asphyxia if they had fetal distress, were depressedat birth, and exhibited a metabolic acidosis [46].

ISSN 2515-8260 Volume 09, Issue 03, 2022

Creatine kinase muscle brain fraction (CK-MB)

CK, also known as creatine phosphokinase (CPK) or phospho-creatine kinase is an enzyme (EC 2.7.3.2) expressed by various tissues and cell types. CK catalyses the conversion of creatine and consumes ATP to create phosphocreatine (PCr) and Adenosine - 5´- diphosphate (ADP). This CK enzyme reaction is reversible, such that also ATP can be generated from PCrand ADP (Figure:4).

Figure 1: Showing the catalytic reaction of Creatine kinase.



In tissues and cells that consume ATP rapidly, especially skeletal muscle,but also brain, photoreceptor cells of the retina, hair cells of the inner ear, spermatozoa and smooth muscle, PCr serves as an energy reservoir for the rapid buffering and regeneration of ATP in situ, as well as for intracellular energy transport by the PCr shuttle or circuit. Thus CK is an important enzyme in such tissues [82].

Types

Various outcomes in asphyxia are

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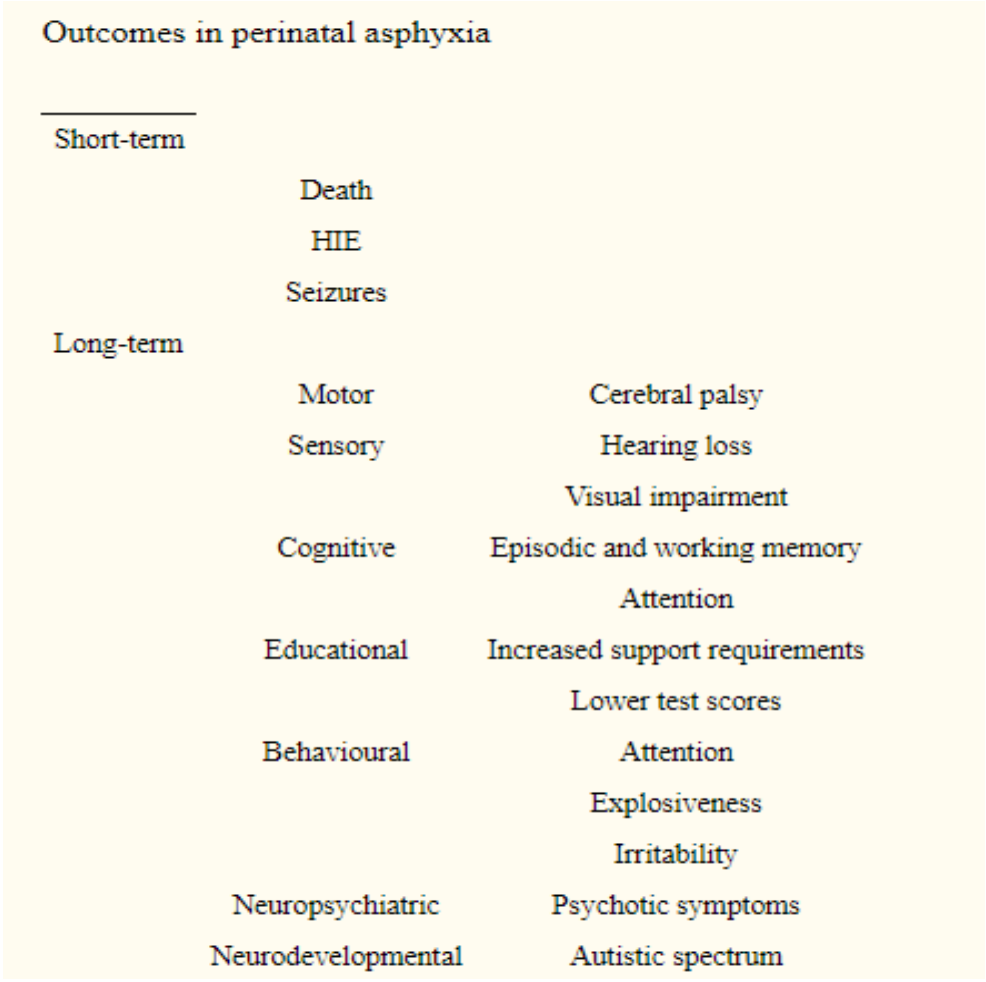
Figure 2: Outcomes in Perinatal aasphyxia



Therapeutic Hypothermia[88]

Three large clinical trials have recently demonstrated that moderate prolonged hypothermia for term neonates with perinatal asphyxia can be an effective neuroprotective strategy (Gluckman et al., 2005; Shankaran et al., 2005; Azzopardi et al., 2009)[90-92]. The National Institute for health and Clinical Excellence (NICE, United Kingdom), as well as the International Liaison Committee on Resuscitation guidelines (ILCOR) recently supported a fullclinical implementation of hypothermia within term asphyxiated newborns.

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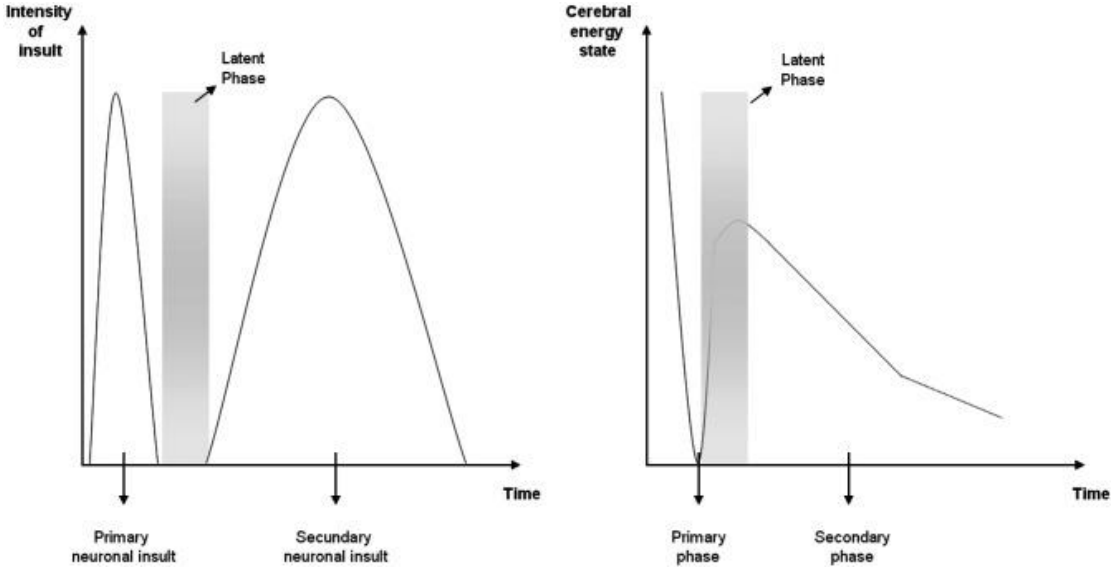
Figure 3: Phases of hypoxia-ischemia



1. The actual episode of hypoxia-ischemia is called ―the primary phase‖ of cell injury. During this phase of energy failure, one observes reduced cerebral concentrations of high-energy phosphorylated compounds such as adenosine triphosphate (ATP) and phosphocreatine. This energy failure results in hypoxic depolarization of cells, loss of membrane ionic homeostasis leading to severe cytotoxic oedema, as well as accumulation of excitatory amino acids(excito-toxins).
2. Following cerebral reperfusion, i.e. the restoration of cerebral circulation and energy state during resuscitation, the cytotoxic oedema may resolve over approximately 30 to 60 minutes, with a partial recovery of cerebral oxidative metabolism in ―the latent phase‖. Although its duration is not precisely known for human infants, animal data suggests that this latent phase lasts in the order of hours.
3. Approximately 6 to 15 hours later, the infant may further deteriorate. This so-called ―secondary phase‖ of energy failure may last several days and is likely to involve multiple patho-physiologic processes such as a further release of excitatory amino acids, free radical formation, a parallel rise in intracerebral lactate, induction of apoptosis, and inflammatory activation, leading to delayed onset of seizures (secondary cytotoxic oedema).

The existence of a time window of recovering cerebral energetics (i.e. the latent phase) led to the introduction of a new therapeutic intervention (i.e. hypothermia) following resuscitation, with the aim to reduce secondary energyfailure after an acute ischemic event. It is uncertain as to how much hypothermia may be effective in situations where chronic antenatal hypoxia/ischemia contributes to the insult.

In all above mentioned trials patients were randomized within 6 hours of age, and half of them were cooled for 72 hours, followed by rewarming at a rate of 0.5°C/hour. A meta-analysis of the 3 trials (Edwards et al., 2010) estimated a risk ratio of 0.81 (95% confidence interval, 0.71- 0.93; P = .002) for the combined rate of death and severe disability, with a number needed to treat of

The meta-analysis also showed that hypothermia resulted in an increase of normal survival, i.e. survival without cerebral palsy and with mental development index and psychomotor developmental index > 84 and normal vision and hearing (risk ratio, 1.53; 95% CI, 1.22-1.93; P < .001), with anumber needed to treat of 9.

Modest hypothermia is a non-specific neuroprotective therapy. It reduces the extent of brain injury via at least the following three mechanisms:

Firstly, hypothermia results in a graded reduction in cerebral metabolism that slows cell depolarization, reduces accumulation of excito-toxic neurotransmitters, and suppresses oxygen free radical release as well as lipid peroxidation of cell membranes.

Secondly, more and more data suggest that hypothermia has a particular role in suppressing apoptotic processes in the developing brain (i.e. programmed cell death), most likely via inhibition of the caspase enzymes, i.e. a large family of enzymes that amplify the intra-cytoplasmic phase of apoptosis after ischemia.

Thirdly, there is good evidence that cooling can suppress the release of pro-inflammatory cytokines and interleukins, reducing direct neurotoxicity, such via suppression of microglial activation.

Hypothermia is increasingly being validated using MRI brain scanning, especially diffusion

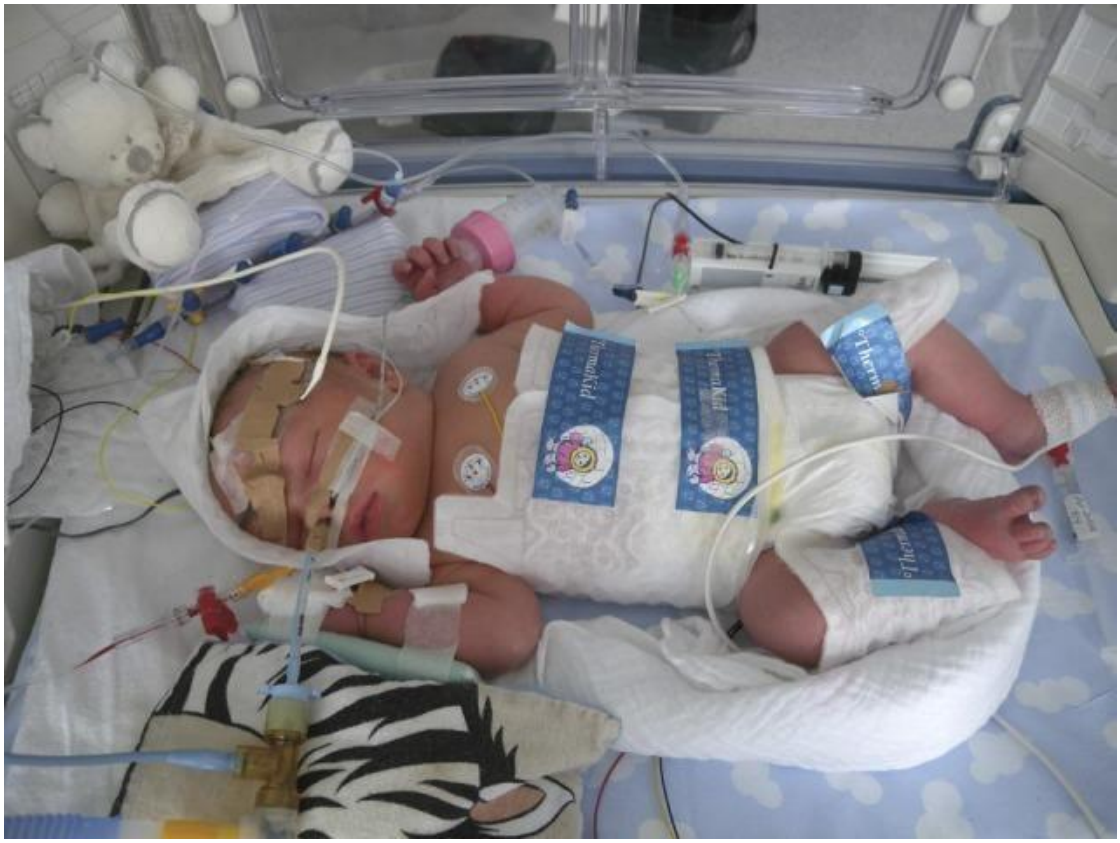
weighted imaging, facilitating early identification of injury. The treatment clearly results in less severe cortical and deep gray nuclear injury (Bonifacio et al., 2011)[93]. Many countries and individual hospitals have therefore now introduced hypothermia as a standard of care for term asphyxiated infants.

Based on the available evidence and the known gaps in our knowledge at the current time, therapeutic hypothermia, if offered, should be executed using a published protocol.

To provide adequate neuroprotection with minimal risk of systemic adverse effects, ideally the brain only should be cooled. In the multi-center Cool Cap Study (Gluckman et al., 2005), involving n = 243 infants with moderate to severe HIE, the scalp and underlying brain tissue were selectively cooled using a CoolCap®, whilst the body was warmed with an overhead heater. However, in view of a temperature gradient between the cerebral cortex and the deep grey nuclei, i.e. structures that are often affected in acute asphyxia, mild systemic hypothermia (34.5°C) was aimed for in order to limit the steepness of the intra-cerebral gradient. This technique is clearly impractical for routine practice.

A second (Shankaran et al., 2005) and third (Azzopardi et al., 2009) large clinical trial successfully used whole body hypothermia, with a target temperature between 33 and 34°C. Although seemingly simple to implement, the lowering and maintenance of the core temperature during 72 hours is complex. Different commercial devices are now in use for whole body hypothermia, with servo-controlled systems aimed at reducing fluctuations in body temperature, being most desirable. Such systems are also helpful in avoiding hyperthermia during rewarming after hypothermia.

Figure 4: Whole Body Cooling



SPECIFICS OF CLINICAL CARE DURING HYPOTHERMIA

Cardio-Pulmonary issues

After asphyxia, hypotension and pulmonary hypertension are frequent clinical findings. Mild hypothermia in itself does not further compromise this cardio- pulmonary instability:

- A temporary physiological reduction in left ventricular cardiac output is seen in some neonates, which disappears during re-warming. Compensatory adjustments in systemic vascular resistance aim to maintain the infant‘s blood pressure within normal range. If insufficient, then inotropic support may be added.
- Sinus bradycardia and prolongation of the QT interval are usually noted. Hence, a heart rate within the normal range may indicate stress and a need for deeper sedation.
- Increased pulmonary vascular resistance (i.e. transient increase in tricuspid regurgitation) can incidentally be seen. However, the multicenter trials of hypothermia (Azzopardi et al., 2009; Gluckman et al., 2005; Shankaran et al., 2005) do not report an increase in the incidence of death due to persistent pulmonary

hypertension. If pulmonary hypertension occurs, then nitric oxide can be used, but hyperoxia should be avoided.

– When lowering the body temperature, blood is more viscous and the solubility of the gases in the blood increases; for example, in vivo values at 33.5°C of $PaCO_2$ are approximately 0.83 the value read at 37°C. Correcting for temperature may result in an increase in $PaCO_2$ with a resultant increase in cerebral blood flow, whereas not correcting may resultin the opposite effect, i.e. hypocapnia-induced vasoconstriction.
– During hypothermia, there may be an increased risk of endotracheal tube obstruction due to sticky secretions. Avoid such by setting the temperature of the humidifier at 37°C.

Drugs

- Stress during cooling should be avoided, since it may reduce the neuroprotective effects of hypothermia. Therefore (dia)morphine should be given to neonates during hypothermia.
- The dosage of (dia) morphine, as well as other drugs such as anticonvulsants and aminoglycosides, should be adjusted during hypothermia, in order to avoid toxic levels due to hypoxic-ischemic injury ofliver and kidneys.

Haematology

– Thrombocytopenia ($< 150 \times 10^9$ /L) can be noted during hypothermia. However, major coagulation disorders and haemorrhage are not reported more often.

Temperature issues

Continuous rectal temperature monitoring is imperative during hypothermia, since active cooling without monitoring of the core temperature carries a risk of overcooling (Kendall et al., 2010). Skin temperature should not be used, as there is a wide discrepancy between skin and rectal temperatures, possibly due to cutaneous vasoconstriction and environmental temperature.

On rewarming (0.5°C/h) special attention should be paid to avoid hyperthermia (> 38°C), since hyperthermia may decreaseneuroprotective effects; therefore, it is advisable to keep the rectal probe in place for 24 hours after rewarming, aiming for a rectally measured temperature of 36.0-36.5°C, along with keeping the infant‘s head uncovered to allow for natural selective head cooling.

Generalised edema is often seen after severe asphyxia. Avoid decubitusand subcutaneous fat necrosis during hypothermia.

Many studies[89] have found that therapeutic hypothermia is feasible and safe even in low to middle income countries with application of newer technology like Phase changing material for cooling. Though it is a standard protocol in most developed nations to use therapeutic hypothermia in birth asphyxia patients, newer studies have established their role in developing nations as well in all level 3 NICUs.

Newer Markers Being Investigated For Prediction Of Asphyxia Outcome

There is increasing interest in the possibility of developing more accurate, early and reliable markers for predicting long term outcome in HIE. These bio- and physiomarkers may take the form of physiological monitoring [EEG and heart rate variability (HRV)], neuroradiological, or biochemical. In fact the idealmarker may be a combination of many of these.

Reduced HRV has shown potential for the assessment of HIE severity and the prediction of long term outcomes.

Radiologically improvements in magnetic resonance imaging has improved our ability to delineate patterns of injury and thereby, aid in prognosis. Piglet models of phosphorous-Magnetic resonance spectroscopy profiles within the first 2 h post-injury can predict the evolution of injury severity.[94]

Blood biomarkers have also shown promise in predicting injury severity and outcome. Although no definitive blood biomarker has entered into routine clinical use, there are a number which have shown promise based on pilot work in small cohorts. Protein markers, such as UCH-L1, IL-6 and IL-16 and Activin A are altered significantly in cord blood taken at birth from infants with HIE[95-97]. In addition GFAP and S100B have shown elevations slightly later, reaching a peak at 24 h.[98]Animal and, more recently, human studies have shown significant alterations in the metabolomic profile of infants with HIE[99-101].Transcriptomics has also shown promise in differentiating infants with perinatal asphyxia

and HIE.[102]Some evidence is also available showing that circulating microRNAs in maternal blood may be useful for the detection of hypoxia in the intrapartum period.[103] Other bodily fluids such as urine and CSF have also been the subject of biomarker discovery work[104].A previous meta-analysis by Ramaswamy et al[105] in this area reported cerebrospinal fluid neuron-specific enolase and IL-1β to be potential markers of abnormal outcome in survivors.

Materials and Methods

Setting:

New born infants admitted to the NICU, department of Pediatrics , KrishnaHospital, Karad during the study period of December 2017 to May 2019

Type - Prospective Observational Study

Study Period:

1 December 2017 to 30 September 2019

Method of Collection of Data (including sampling procedure if any): Cases were the new born infants admitted to the NICU, KrishnaHospital, Karad.They were studied from 1 December 2017 - 31 May 2019.

Sample Size : n= [4 * (SD)squared] / (x * ε)squaredSD-standard deviation

x- mean

ε- precision

Using above formula, sample size calculated was 55 cases at precision ε= 0.4based on the study by Shylaja et al in 2014.

Inclusion Criteria:

Study group: New born infants with signs of birth asphyxia with

1. APGAR score <=7 at 5 min
2. In case of outborn patients,when APGAR score was not known - clinical evidence of multiorgan system dysfunction like oligo-anuria, congestive heart failure not related to structural defects, shock, ventilatory dependence or requirement of increased oxygen for more than 24 hours, elevated transaminases, DIC, Necrotising enterocolitis etc.
3. All term neonates (≥37 weeks of gestation) having birth weight (≥1.5kg)

Exclusion criteria: Patients with -

1. Gestational age of <37 completed weeks
2. Very Low birth weight babies (<1.5 kg)
3. Major congenital malformations
4. Chromosomal abnormalities
5. Metabolic disorders
6. Congenital infection
7. Birth trauma
8. Septic shock
9. Full-term newborns with severe jaundice, severe septicemia, congenital anomalies of the hepatobiliary system
10. Babies undergoing potentially hepatotoxic drug therapy

Method of examination:

- Project was approved by institutional ethical committee before conduction.Written informed consent was obtained from parents/guardians prior to enrolment of subjects in study.
- At birth,all the babies fulfilling inclusion criteria were admitted to NICU where detailed examination was done by same assessor for all the patients.All relevant history and clinical findings as per proforma were noted.
- Babies were grouped according to Sarnat and Sarnat stages of HIE as Stage I, II, and III.
- The biochemical analysis for the parameters, that is LDH, CK-MB, was be done using reagent kits and Auto Analysers– like TOSOH AIA-360 in the biochemistry laboratory of the institute.
- 1 ml venous blood was collected under aseptic precautions in a plain bulb for testing levels of CKMB at 8 hours, 24 hours and LDH at 72 hours after birth. The levels were compared against normal reference values mentioned in standard published literature.(upper limit of 4.5 ng/ml for CKMB at 8 and 24 hours and upper limit of 580 U/L for LDH at 72 hours).

LDH at 72 hours less than mean of 1474U/L while 19 cases had value greater than mean.

-Among patients who were classified as HIE 2(10),2 had values less than mean while 8 had values greater than mean.

-Among patients who were classified as HIE 3(5) ,no cases had value of LDH at 72 hours less than mean of 1474U/L while 5 cases had value greater than mean.

-Thus as LDH levels at 72 hours increased,the severity of HIE was greater and this correlation was significant with chi square value of 7.426 and p-value of .024*

Table 3: Correlation between CKMB at 8 hours and outcome

Outcome	CKMB at 8 Hours (ng/ml)		Total	Chi Square Value
	<25.7	>25.7		12.327
DISCHARGED WITHOUT NEURODEFICIT	41(85%)	7(15%)	48	p value
DISCHARGED WITH NEURODEFICIT	1(50%)	1(50%)	2	.002*
EXPIRED	1(20%)	4(80%)	5	
	43	12	55	

-Among patients discharged without neurodeficit(48),41 had CKMB at 8 hoursless than mean of 25.7ng/ml and 7 had values greater than mean.

-Among patients discharged with neurodefici t(2),1 had CKMB at 8 hours lessthan mean and 1 had value greater than mean.

-Among patients who expired (5),1 had CKMB at 8 hours less than mean and4 had values greater than mean.

-Thus, as CKMB at 8 hours increased the outcome was worse and thiscorrelation was significant with chi square value 12.327, p value – 0.002*.

Table 4: Correlation between CKMB at 24 hours and outcome

Outcome	CKMB 24 Hours (ng/ml)		Total	Chi square value
	<33	>33		9.54
DISCHARGED WITHOUT NEURODEFICIT	35(73%)	13(27%)	48	p value
DISCHARGED WITH NEURODEFICIT	0	2(100%)	2	.008*
EXPIRED	1(20%)	4(80%)	5	
	36	19	55	

-Among patients discharged without neurodeficit (48),35 had CKMB at 24hours less than mean of 33ng/ml and 13 had values greater than mean.

-Among patients discharged with neurodeficit (2),none had CKMB at 24 hours less than mean and 2 had value greater than mean.

-Among patients who expired(5),1 had CKMB at 24 hours less than mean and

4 had values greater than mean.

-Thus, as CKMB at 24 hours increased the outcome was worse and this correlation was significant with chi square value 9.54,p value – 0.008*.

Table 5: Correlation between LDH at 72 hours and outcome

Outcome	ıt 72 hours(U/L)		Total	Chi square value
	<1474	>1474		3.967
DISCHARGED WITHOUT NEURODEFICIT	22(46%)	26(54%)	48	p value
DISCHARGED WITH NEURODEFICIT	1(50%)	1(50%)	2	.138
EXPIRED	0	5(100%)	5	
	23	32	55	

-Among patients discharged without neurodeficit(48),22 had LDH at 72 hoursless than mean of 1474 U/l and 26 had values greater than mean.

-Among patients discharged with neurodeficit(2),1 had LDH at 72 hours lessthan mean of 1474 U/l and 1 had value greater than mean.

-Among patients who expired(5),none had LDH at 72 hours less than mean of1474 U/l and 5

had values greater than mean.

-Thus, as the LDH at 72 hours increased the outcome was worse but thiscorrelation was not significant with chi square value 3.967,p value – 0.138.

Table 6: Comparative study of complications in cases:

COMPLICATIONS		Reddyet al	Karunatilaka DH et al	HM Sanjayet al	Rajakumaret al	Current study
HIE	Mild		14%	6%	27%	73%
	Moderate		9%	24%	60%	18%
	Severe		3%	8%	13%	9%
Respiratory distress (RD)				76%	67%	27%
Acute renal failure (ARF)						3.6%
Shock		16%		12%	17%	11.5%
Inotrope support		16%				11.5%
Congestive cardiac failure (CCF)				2%	37%	1.8%
Necrotizing enterocolitis (NEC)						1.8%
Hypotonia		68%		38%	73%	73%
Death				10%	17%	9.1%

In the current study the distribution of HIE was 73% with mild asphyxia,18% with moderate asphyxia and 9 % with severe asphyxia. Thus, majority hadmild HIE unlike in the study by Rajakumar et al where although 100% patients developed HIE, majority (60%) had moderate asphyxia.

In the study by HM Sanjay et al,38% had HIE out of which maximum(24%) had moderate HIE.

Respiratory distress was present in 27% of cases in this study compared to 76% and 67% respectively in studies by HM Sanjay et al and Reddy et al respectively.

Shock developed in 11.5 % of the patients in this study, with similar number instudies by HM Sanjay et al (12%), Reddy et al(16%) and Rajakumar et al(17%). Congestive cardiac failure occurred in 1.8% of the patients in our study, similar to findings of Sanjay et al

(2%) but much lesser than that observed in study by Rajakumar et al (37%).

Hypotonia was seen in 73 % of the cases in this study similar to that in studiesby Rajakumar et al (73%),Reddy et al(68%) but lesser than that in study by Sanjay et al(38%). Death occurred in 9.1 % patients of our study similar to proportion in the study by Sanjay et al (10%). Differences observed in our study may be attributed to different inclusion criteria used in different studies, different grading systems used, differences in resuscitative measures used, post asphyxia monitoring, etc.

Summary

- In our prospective observational study we studied 55 neonates withperinatal asphyxia who fulfilled the inclusion criteria.
- Detailed history was noted and clinical examination was done as perproforma.
- Venous samples were assessed for CKMB at 8 hours and 24 hours and LDH at 72 hours. Neonates were followed up until discharge and any relevant observations during course of stay and outcome were noted.These neonates were classified into 3 groups based on the stage of HIE they developed.
- Statistical analysis was done to find any correlation between increase in enzyme levels and severity of HIE,outcome of patient and the requirement for intensive procedures.We found that

➢ CKMB and LDH levels are elevated in patients of birth asphyxia

➢ There is a correlation between increased enzyme levels and

- Severity of HIE
- Outcome of these patients
- Need for intensive procedures

- Thus we found that enzyme levels of CKMB and LDH can be used to diagnose perinatal asphyxia and differentiate between asphyxiated andnon- asphyxiated babies.
- Mainly,the elevated enzyme levels can be utilised to predict the outcome and prognosis of birth asphyxia patients.This will help us to give guarded prognosis to caregivers of children whose enzyme values are markedly raised .
- Enzyme estimation has greater significance in developing countries like ours,where adequate birth history may not be available regarding resuscitation methods used at delivery ,especially in the periphery.

- Tests to detect raised enzyme levels are economical and do not require sophisticated equipment or advanced technical expertise unlikesome other tests used to predict the outcome in perinatal asphyxia patients like -MRI scan, newer protein markers ,etc.
- Neuroprotective measures like therapeutic hypothermia(TH) can be initiated for asphyxiated neonates with raised enzyme levels. Although our study assessed first enzyme levels at 8 hours after birth and TH should ideally be started within 6 hours of birth,studies[106] have shown that even late TH within the first 24 hours may offer a modest protective benefit against severe neurodevelopment disability.

❖ **Limitations of this study-**

Sample size was fairly small and it was an observational study. Large Multi centre case control studies and trials will be required to more definitively establish the role of enzyme markers as predictors of the presence of asphyxia, it‘s severity and outcome or prognosis. The cases of this study were followed only until discharge and longer follow up would give a better idea about long term neurodevelopment disabilities.

Conclusion

- CKMB levels at 8 hours, 24 hours and LDH levels at 72 hours after birth are elevated in patients of perinatal asphyxia.
- There is a correlation between increased enzyme levels and
  - Severity of HIE
  - Outcome of these patients
  - Need for intensive procedures
- Thus, enzyme levels of CKMB and LDH can be used to diagnose perinatal asphyxia, especially in cases where proper birth history is not available.
- Mainly, the elevated enzyme levels can be utilised to predict the outcome and prognosis of perinatal asphyxia patients. This will help us to give guarded prognosis to caregivers of children whose enzyme values are markedly raised .
- Neuroprotective measures like therapeutic hypothermia can be initiated in neonates having raised enzyme levels.

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