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Perinatal Asphyxia and Enzyme Markers LDH and CKMB

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Abstract

The neonatal mortality rate (NMR) decreased from 52 per 1000 live births in 1990 to 28 per 1000 live births in 2013[1].The slower decline has led to increasing contribution of neonatal mortality to infant and under-five mortality. Among neonatal deaths, the rate of decline in early neonatal mortality rate (ENMR) is much lower than that of late NMR. Similarly, the perinatal mortality is also still fairly high. The rate of decline in NMR, and to an extent ENMR,has accelerated with the introduction of National Rural Health Mission in mid- 2005. Almost all states have witnessed this phenomenon, but there is still a huge disparity in NMR between and even within the states. The disparity is further compounded by rural–urban, poor–rich and gender differentials. There is an interplay of different demographic, educational, socioeconomic, biological and care-seeking factors, which are responsible for the differentials and the high burden of neonatal mortality. Addressing inequity in India is an important cross-cutting action that will reduce newborn mortality.

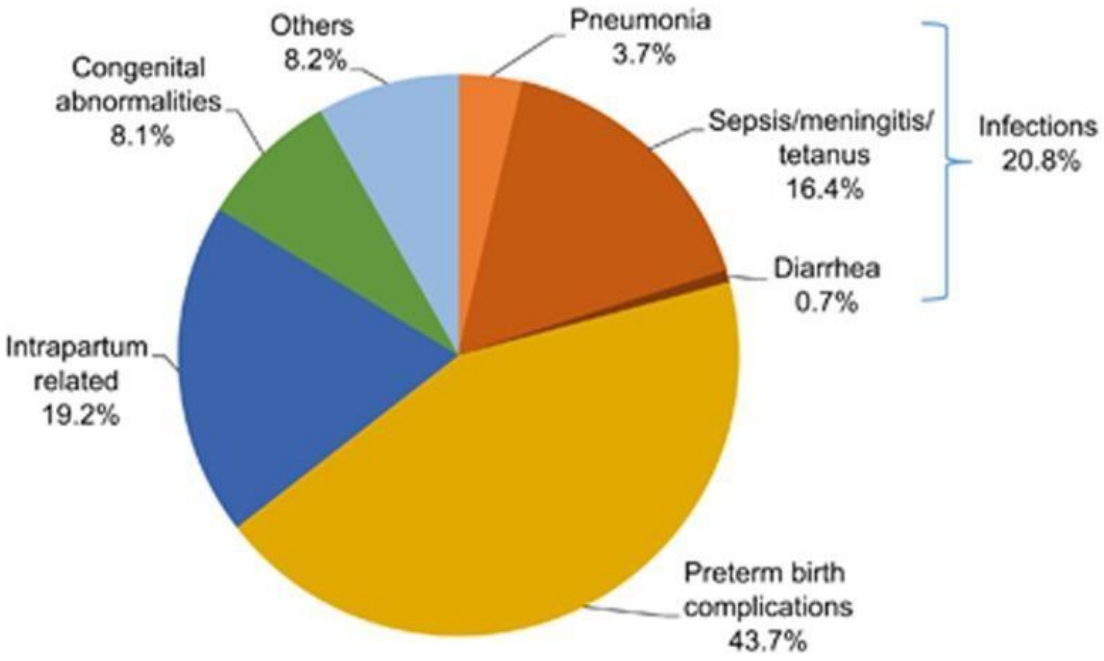
The neonatal period—the first 28 days of life—carries the highest risk of mortality per day than any other period during the childhood. The daily risk of mortality in the first 4 weeks of life is ~30-fold higher than the post-neonatal period, that is, from 1 month to 59 months of age. Still, newborn health did not receive the commensurate attention it deserved until during the past decade. This has resulted in a slow decline in neonatal mortality rate (NMR) in most countries including India, and has hampered their achieving the Millennium Development Goal-4 (MDG 4) by year 2015.

Introduction

India contributes to one-fifth of global live births and more than a quarter of neonatal deaths. Given the early NMR of 22 per 1000 live births, deaths in the first week alone account for ~45% of total under-five deaths. Obviously, the

Committing to Child Survival: A Promise Renewed' goal of reducing under- five mortality to 20 or less per 1000 live births by 2035[2] will not be attained without specific efforts to reduce newborn mortality.

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. These findings are very similar to the overall global pattern.



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%.

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.

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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.

Pathophysiology of HIE[4]

The neuropathology of this injury includes three principal lesions: selective neuronal necrosis, parasagittal cerebral injury, and cerebral white matter injury. Several recurring pathogenetic themes include initiating factors, principally ischemia, impinging on specific regional anatomical and metabolic/cellular characteristics that underlie a maturation-dependent vulnerability. Ischemia is linked to deleterious perinatal events and to impaired cerebrovascular autoregulation. Selective neuronal necrosis, the most common variety of injury, refers to necrosis of neurons in a characteristic distribution. Three basic patterns are: Diffuse neuronal injury (cerebral cortical, deep nuclear, brain stem) occurs with very severe, relatively prolonged insults; a cerebral cortical–deep nuclear neuronal predominance occurs with moderate to severe, relatively prolonged insults; deep nuclear– brain stem neuronal predominance occurs with severe, relatively abrupt insults. The principal pathogenetic themes are ischemia and regional metabolic and cellular factors. The most important of the latter is the regional distribution of excitatory amino acid receptors. Parasagittal cerebral injury refers to necrosis of neurons and pre-oligodendrocytes in a characteristic distribution, particularly involving superomedial aspects of the cerebral cortex. The principal pathogenetic themes are ischemia, regional vascular factors, and regional cellular/metabolic factors. Regarding vascular and cellular factors, the lesions occur in the distal fields and border zones of major cerebral vessels, and affected neurons and pre-oligodendrocytes exhibit exuberant expression of excitatory amino acid receptors. Cerebral white matter injury is similar, albeit less severe, to the white matter injury of premature infants. The principal pathogenetic themes are ischemia and intrinsic vulnerability of pre-oligodendrocytes.

Clinical evaluation and laboratory values are used to assess and manage the asphyxiated babies. The common indicator for intrapartum asphyxia is severe metabolic acidosis (pH <7 in umbilical artery cord blood).Cranial and doppler USG, CT imaging and MRI are most common imaging techniques.

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.

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.

Since the goal of observed pathophysiology should be prevention, based on the understanding of the involved mechanisms, a variety of markers have been examined to identify perinatal hypoxia including electronic fetal heart monitoring, intrapartum fetal scalp blood pH, low APGAR scores, cord pH, EEG, CT and MRI scans and Doppler flow studies. The current problem, from a historical perspective, then becomes our inability to precisely distinguish the false positive affected from the true positive asphyxiated or compromised fetus. [9]

Review of Literature

Several studies have been conducted to evaluate better markers that help distinguish an asphyxiated from a non- asphyxiated neonates and study any correlation between enzyme levels and severity of HIE, complications, outcome, intensive procedure requirement, etc..

In a study by **Primhak et al** [10]in 1985, 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.

In 1990 **Sanchez-Nava et al** [11]showed that AST, ALT and LDH were raised among asphyxiated babies.

In 2005, **Boo NY et al**[17]showed that at birth, asphyxiated infants had significantly higher concentrations of cTnT and CK-MB than controls. Unlike CK- MB, serum cTnT concentrations were significantly higher in asphyxiated infants who died or developed cardiac dysfunction.

In 2008 **Reddy S et al**[18]concluded that raised LDH had 100% sensitivity,while CK-MB had 100% specificity for asphyxia. They found that LDH at 72 hoursof life is the most accurate at differentiating asphyxiated from non- asphyxiated symptomatic neonates and that LDH could be used at 3 days of age to diagnose asphyxia retrospectively.

In a study by **Rajakumar PS et al** [19]in 2008, the cardiac enzymes, cTnT and CK-MB were significantly elevated in cases when compared with controls. The mean CK-MB levels among cases and controls were 121±77.4 IU/L and 28.8±20.2 IU/L respectively. The specificity and sensitivity of CK-MB were 56.5% and 75.7% respectively.

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.

Perinatal asphyxia

Birth asphyxia is the most common and important cause of preventable cerebral injury occurring in the neonatal period, but although asphyxia at birth is a commonly made diagnosis, there is no universally accepted definition for it. Asphyxia, at a pathophysiological level, is the simultaneous combination of both hypoxia and hypoperfusion, which impairs tissue gas exchange leading to tissue acidosis. In current clinical usage there remains variability in both the meaning and interpretation of the term ‗birth asphyxia'. Hence when determining the incidence, etiology and outcome of birth asphyxia there is wide variation. Many have suggested that this term should no longer be used . Since there is simultaneous occurrence of hypoxia and ischaemia, the term hypoxic-ischaemic insult is now preferred.

Undoubtedly hypoxia-ischemia (HI) can lead to severe brain injury but a major concern regarding the term is in those children who develop long term neurodisability such as cerebral palsy. In these children there is an often false assumption that they were ‗injured' during events of labour and delivery with the result that obstetricians and midwives are targeted as the person responsible for those neurologic injuries. In resource rich countries two of every 1000 live born children develop cerebral palsy (CP). Evidence suggests that 70-80% of these CP cases are due to prenatal factors and that birth asphyxia plays a relatively minor role (<10%) [38].

Birth asphyxia is defined by the World Health Organization (WHO) as ―the failure to initiate and sustain breathing at birth.‖. The ICD-10 definition of birth asphyxia is dependent on the APGAR score at 1 min of age.

An APGAR score at 1 min of 0-3 defines severe birth asphyxia and an APGAR score of 4-7 defines moderate asphyxia.

The NNPD 2000 used a similar definition for perinatal asphyxia and defined moderate asphyxia as slow gasping breathing or an APGAR score of 4-6 and severe asphyxia as no breathing or an APGAR score of 0-3 at one minute of life.

The National Neonatology Forum of India has defined asphyxia as ―gasping or ineffective breathing or lack of breathing at one minute of life‖ Newer terms include ‗birth depression', which is a descriptive term to indicate a newborn with poor APGAR but without passing judgement on etiology. The use of word prenatal rather than 'birth' supports the pathological processes that may begin many hours before birth and continue for many hours afterwards. There are numerous causes, and the clinical manifestations vary. Infants who experience mild asphyxia may show no neurological injury. However, severe asphyxia may be fatal in utero, or immediately after birth,with survivors showing extensive neurological sequelae, with or without cognitive deficits.

The following terms may be used in evaluating a term infant at risk for brain injury in the perinatal period [39]:

A. Neonatal depression

It is a general term used to describe an infant who has a prolonged transition from an intrauterine to an extrauterine environment. These infants usually have low 1- and 5-minute APGAR scores.

B. Neonatal encephalopathy

It is a clinical term used to describe an abnormal neurobehavioral state that consists of a decreased level of consciousness with abnormalities in neuromotor tone. It characteristically begins within the first postnatal day and may be associated with seizure-like activity, hypoventilation or apnea, depressed primitive reflexes and the appearance of brain stem reflexes. It does not imply a specific etiology, nor does it imply irreversible neurologic injury.

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C. Hypoxic-ischemic encephalopathy (HIE)

It is an abnormal neurobehavioral state in which the predominant pathogenic mechanism is impaired cerebral blood flow.

D. Hypoxic-ischemic brain injury

It refers to neuropathology attributable to hypoxia and/or ischemia as evidenced by biochemical (such as serum creatine kinase brain bound [CK-BB]), electrophysiologic (EEG), neuroimaging (cranial ultrasonography,MRI, CT), or postmortem abnormalities.

Incidence.

The frequency of perinatal asphyxia is approximately 1% to 1.5% of live births in the Western Hemisphere and is inversely related to gestational age and birth weight[42]. It occurs in 0.5% of live born infants >36 weeks gestation and accounts for 20% of perinatal deaths (50% if stillborns are included). A higher incidence is noted in term infants of diabetic or toxemic mothers, infants with intrauterine growth restriction, breech presentation, and postdated infants. In India, 8.4% of inborn babies have a one minute APGAR score less than 7and 1.4% suffer from HIE[43].

Etiology

In term infants, 90% of asphyxial events occur in the antepartumor intrapartum period as a result of impaired gas exchange across the placenta that leads to the inadequate provision of oxygen (O_2) and removal of carbon dioxide (CO_2) and H^+ from the fetus. The remainder of these events occurs in the postpartum period and is usually secondary to pulmonary, cardiovascular, or neurologic abnormalities [44].

A. Factors that increase the risk of perinatal asphyxia include the following:

1. Impairment of maternal oxygenation.
2. Decreased blood flow from mother to placenta.
3. Decreased blood flow from placenta to fetus.
4. Impaired gas exchange across the placenta or at the fetal tissue level.
5. Increased fetal O_2 requirement.

B. Etiologies of perinatal hypoxia-ischemia include the following:

1. Maternal factors: hypertension (acute or chronic), infection, diabetes, hypotension, vascular disease, drug use, and hypoxia due to pulmonary, cardiac, or neurologic disease.
2. Placental factors: infarction, fibrosis, abruption, or hydrops.
3. Uterine rupture.
4. Umbilical cord accidents: prolapse, entanglement, true knot, compression.
5. Abnormalities of umbilical vessels.
6. Fetal factors: anemia, infection, cardiomyopathy, hydrops, severe cardiac/ circulatory insufficiency.
7. Neonatal factors: severe neonatal hypoxia due to cyanotic congenital heart disease, persistent pulmonary hypertension of the newborn, cardiomyopathy, other forms of neonatal cardiogenic and/or septic shock.

Clinical Features after Birth

Hypoxic-ischaemic encephalopathy (HIE)

Neonatal encephalopathy refers to abnormal neurological behaviour in the neonatal period and may be caused by a wide range of conditions. If the full-term brain has been compromised by an hypoxic-ischemic event (asphyxia) during delivery, it is likely that the infant will show a disturbance in neurological behavior, a state referred to as HIE. It is unclear to what extent preterm babies can manifest similar clinical features following hypoxic- ischaemic injury compared to term babies . Infants show a sequence of often transient encephalopathic behavior lasting often for days which is dependent on the severity and duration of the asphyxial event. Grading systems have been published to define the degree of encephalopathy. Sarnat and Sarnat[63] introduced a grading system to describe the neurological abnormality, which they referred to as HIE (Table:3) and which has been modified by Levene

MI [64](Table:4).

Table 1: Sarnat and Sarnat Stages of Hypoxic-Ischemic Encephalopathy

Severity	Stage 1 (Mild)	Stage 2 (Moderate)	Stage 3 (Severe)
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
Pupils	Mydriasis	Miosis	Variable
Heart Rate	Tachycardia	Bradycardia	Variable
Seizures	None	Common	Uncommon

Table 2: A clinical grading system for hypoxic-ischaemic encephalopathy by Levene MI. [64]

Mild (grade I) encephalopathy

This stage is characterised by hyperalertness, staring (decreased frequency of blinking), normal or decreased spontaneous motor activity and a lower threshold for all stimuli, including the easily elicited Moro reflex. Seizures are not a feature.

Moderate (grade II) encephalopathy

Seizures occur commonly. There is lethargy, hypotonia with reduced spontaneous movements, a higher threshold for primitive reflexes, and mainly parasympathetic responses. A consistent feature is differential tone between the upper and lower limbs, with the arms being relatively hypotonic compared to the legs.

Severe (grade III) encephalopathy

These neonates are comatose, with hypotonia and no spontaneous movements. Primitive reflexes and the suck reflex are often absent. Seizures may be frequent and prolonged, although in the most severe cases there may be no seizure activity and an isoelectric EEG. Asphyxia is not the only cause of neonatal encephalopathy and alternative causes such as hypoglycemia and meningitis must be considered and excluded before HIE can be reliably

used as a feature of postasphyxial insult (Table:5) [41]. In particular, neonatal convulsions alone with clinical interseizure normality are not a feature of HIE, nor is the baby who shows an unchanging pattern of neurological abnormalities in the neonatal period. It has been suggested that in the majority of cases, 'neonatal encephalopathy' in full-term babies may not be due to intrapartum events, but may originate in the antepartum period [65]. The severity of HIE is the best clinical method currently available to predict subsequent outcome following asphyxia, but it has a number of disadvantages.Firstly, the severity of HIE can only be determined retrospectively as the clinicalneurological features of asphyxia take some time to evolve. Secondly, other organs such as the kidneys and heart may be compromised due to asphyxia but the fetus preserves blood flow to the brain thereby sparing cerebral function. The lack of encephalopathy does not necessarily indicate that the infant has not suffered from significant intrapartum asphyxia [41].

Table 3: Differential diagnosis of hypoxic-ischaemic encephalopathy.[41]

Infective	Meningitis (bacterial or viral) Encephalitis (herpes simplex)
Traumatic brain lesion	e.g. Subdural hemorrhage
Vascular	Neonatal stroke Shock secondary to acute blood loss (antepartum/intrapartum)
Metabolic	Hypoglycemia Hypo/hypernatremia Bilirubin encephalopathy
Inborn error of metabolism	Urea cycle defects Pyridoxine dependency Lactate acidemias Amino acidemias (non-ketotic hyperglycemia) Organic acidemias
Congenital brain malformation	e.g. Neuronal migration disorder
Neuromuscular disorder	e.g. Spinal muscular atrophy
Maternal drug exposure	Acute or chronic

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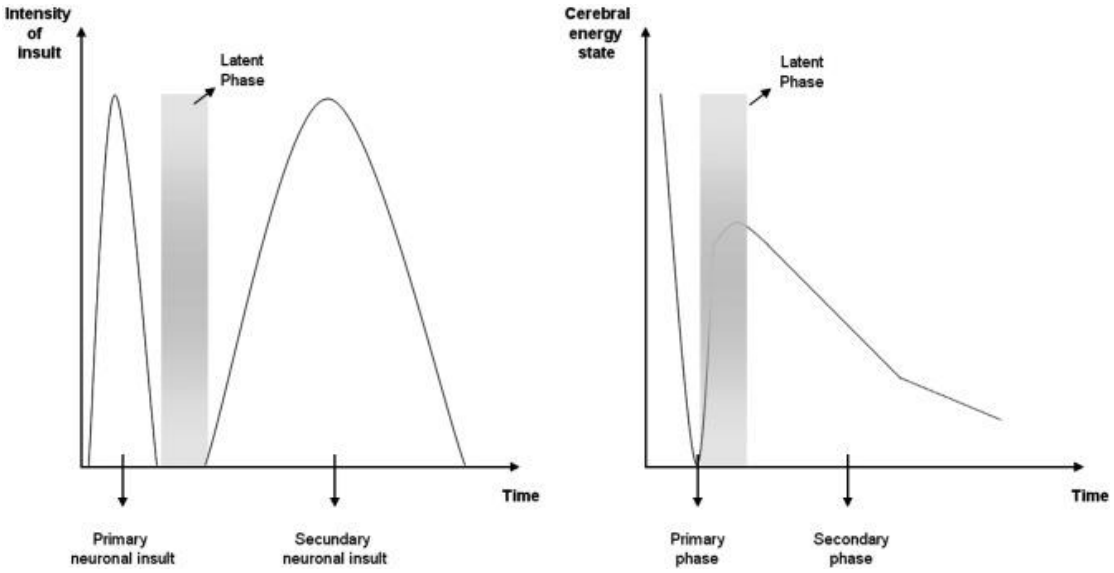
Figure 1: Phases of hypoxia-ischemia



(i) 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).

(ii) 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.

(iii) 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).

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Materials and Methods

Setting:

New born infants admitted to the NICU, department of Pediatrics , Krishna Hospital, 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, Krishna Hospital, 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 -

(i) Gestational age of <37 completed weeks

(ii) Very Low birth weight babies (<1.5 kg)

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(iii) Major congenital malformations
(iv) Chromosomal abnormalities
(v) Metabolic disorders
(vi) Congenital infection
(vii) Birth trauma
(viii) Septic shock
(ix) Full-term newborns with severe jaundice, severe septicemia, congenital anomalies of the hepatobiliary system
(x) 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).

Data Analysis

All data was collected, compiled, and subjected to statistical analysis with thehelp of SPSS software (Version 20.0, IBM).

RESULTS

NST	No. of patients (n)	Percentage (%)
Non-Reassuring	15	27.27
Reassuring	40	72.73
Total	55	100

Among the 55 neonates in our study, 15 showed non reassuring Non Stress Test while 40 patients showed reassuring NST. Non reassuring NST is suggestive of fetal distress.

Table 4: Distribution based on Meconium Stained Amniotic Fluid(MSAF)

MSAF	No. of patients (n)	Percentage (%)
Positive	16	29.1
Negative	39	70.9
Total	55	100

Out of 55 neonates 16 had meconium stained amniotic fluid while 39neonates had clear amniotic fluid.

Table 5: Distribution of complications

Complications	No. of patients (n)	Percentage (%)
HIE	55	100
Respiratory distress (RD)	15	27.27
Acute renal failure (ARF)	02	3.64
Shock	06	11.53
Inotrope support	06	11.53
Congestive cardiac failure(CCF)	01	1.81
Necrotizing enterocolitis(NEC)	01	1.81
Death	05	9.09

All the neonates (100%)had some degree of HIE. 15 (27.27%) patients had respiratory distress, 2 (3.64%) showed acute renal failure, 6 (11.53%) had shock, 6 (11.53%) were given inotropic support, congestive cardiac failure and necrotizing enterocolitis were seen in 1 (1.81%) patient each. Deathcaused by complications occurred in 5(9%) patients.

Birth asphyxia is a major contributor to the burden of neonatal mortality all over the world and even more in developing countries such as India. Due to various efforts of the Government by way of facilities, education, incentives, etc; the percentage of institutional deliveries has greatly increased from 38.7% to 78.9% in the decade to 2015-2016 according to NFHS-4.This number still implies that over 50 lakh births every year are not institutional

and hence , those babies are not receiving optimal care at birth and are highly susceptible to develop multiple complications, birth asphyxia being a major one of them.

In addition to this, in a large number of institutional deliveries as well, aspects of perinatal history such as presence of foetal distress, APGAR score, resuscitation required, etc are not recorded correctly. As a result, in a largenumber of babies presenting to tertiary care centres, exact perinatal history is not known.

In such cases, retrospective diagnosis of birth asphyxia helps in management of complications as well as predicting prognosis of these patients.

A study by Laryea CC et al in 2018 in Africa found that the mean out-of- pocket expenditure for perinatal asphyxia was 132.3 $.This amount assumes higher significance in our country where this will amount to a major portion of the average income of a caregiver.

In a large number of caregivers, basic needs of living are barely met withtheir incomes and bearing the costs of treatment in neonatal period followed by greatly added cost of providing for child with neurological sequelae of birth asphyxia is a matter that requires serious consideration. Withdrawing treatment is a big decision that the caregivers make in such situations and any test that can predict the outcome in such patients will greatly aid in decision making for caregiver as well as healthcare provider.

Various results of our study can be compared with existing studies as follows: Some basic parameters that were studied were-
Gender distribution:

In our study, maximum number of cases, 39 out of 55 (71%) were male .

This finding is similar to that obtained in studies by Reddy et al and HM Sanjay et al[110].
Birth order:

In our study there was apparently not much difference in frequency of birth asphyxia among mothers who were primigravida(45%) and multigravida (55%)
This was similar to findings in studies by Reddy et al and HM Sanjay et al but contradictory

to study by Aslam et al[111] where birth asphyxia was found in higher frequency among primi gravida mothers.

Mode of delivery:

In this study among patients with birth asphyxia frequency of delivery by caesarean section (71%) was higher than delivery per vaginum (25%) or assisted vaginal delivery(4%). This is similar to findings in studies by Reddyet al and HM Sanjay et al but study by Aslam et al didn‘t find any significant increase in frequency of asphyxia in babies delivered by caesarean section.

NST:

In this study , frequency of patients with reassuring NST (73%) was higher than those with non reassuring NST(27%) compared to non reassuring NST being present in 78% cases in study by HM Sanjay et al and 92% in study by Reddy et al.

MSAF:

In this study , frequency of patients with MSAF (29%) was lower than those with clear amniotic fluid(71%) compared to MSAF being present in 64% casesin study by HM Sanjay et al and 8% in study by Reddy et al.

Table 6: Comparative study of complications in cases:

COMPLICATIONS		**Reddy et al**	**Karunatilaka DH et al**	**HM Sanjay et al**	**Rajakumar et 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 studyby 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.

Table 7: Comparative study of CKMB level above cut off in cases at 8 hours

Study	Above cutoff	Below Cutoff
Current study	98%	2%
Reddy et al	36%	64%
Shylaja et al	40%	60%

Table 8: Comparative study of CKMB level above cut off in cases at 24 hours

Study	Above cutoff	Below Cutoff
Current study	100%	-
Reddy et al	36%	64%
Shylajaet al	40%	60%

-

Table 9: Comparative study of LDH above cutoff in cases at 72 hours

Study	Above cutoff	Below Cutoff
Current study	100%	-
Reddy et al	100%	-
Shylaja et al	96%	4%

Among patients showing above cutoff values of CKMB, significantly higher proportion belonged to case group than the control group in studies by Reddy et al ,Shylaja et al.

In our study 98% cases had CKMB at 8 hours above cutoff and 100% had CKMB at 24 hours and LDH at 72 hours above cutoff.

When compared to CKMB at 8 hours in this study ,other studies didn‘t show a higher frequency of above cutoff values among cases such as Reddy et al showing 36%,Shylaja et al showing 40% above cutoff.

However in a study by Chawla et al ,100% of the patients with CKMB above cutoff belonged to the case group ,i.e. had birth asphyxia.

The differences may be due to the fact that our study is an observational study and included only cases unlike the other studies which were case control studies.In addition,all the cases in our study had more severe sequelae of birth asphyxia than these studies which may be the reason for higher enzyme levels in our study.

Similarly, CKMB at 24 hours was above cutoff for 100% patients in our study, but in a lower proportion of cases in studies by Reddy et al (36%) and Shylaja et al (40%).Difference can be explained by similar reasons as above.

In addition, CKMB was measured in units of mass(ng/ml) in our institute and measured in units of activity (IU/L) in most of the reference studies. One of these units cannot be

converted into the other as they measure different variables. Hence, comparison may not be accurate.

Significantly raised CKMB levels at 8,24 hours among patients of birth asphyxia was also found in studies by Nakajima et al, Beken S et al, Chavan et al. As regards LDH at 72 hours,our study showed above cutoff values in 100% patients. This was similar to findings by Reddy et al (100%) and Shylaja et al(96%). Significantly raised LDH levels at 72 hours among patients of birth asphyxia was also found in studies by Patra et al, Nakajima et al, Beken et al, Chavanet al.

In these case control studies, the number of cases with values of LDH above cutoff were significantly greater than the number of controls above cutoff value.

Table 10: Comparative study of mean CKMB level at 8 hours, CKMB at 24hours, LDH at 72 hours

Study	**n CKMB 8hours**	**ean CKMB 24 hours**	**n LDH 72hours**
Current study	25.69+/-36.13 ng/ml	32.96+/-27.67 ng/ml	1474.15+/-586.58 U/L
Reddy et al	176.1+/- 243 U/L	49.6+/-36 U/L	1109.5+/-520.6U/L
Sanjay et al	83.98+/-19.6 U/L	-	555.65+/-105.95U/L
Sadoh et al	2.3+/-2.5 ng/ml		
Vargas et al		36.7 ng /ml	546IU/L

Mean CKMB at 8 hours in this study was well above the cutoff similar to study by Reddy et al but unlike the study by Sanjay et al and Sadoh[112] et al. Difference may be due to greater severity of complications in cases of our study as described.

Mean CKMB at 24 hours was well above cutoff level similar to studies by Vargas et al.

Mean LDH was well above cutoff similar to studies by Reddy et al.

- Correlation between CKMB at 8 hours and severity of HIE

Our study found that there was a significant rise in CKMB at 8 hours withincrease in severity of HIE.

Similar findings were obtained in studies by Rabindran et al,Saha[113]et al,Beken S et al,Ashraf [114]et al.

- Correlation between CKMB at 24 hours and severity of HIE

Our study found that there was a significant rise in CKMB at 24 hours withincrease in severity of HIE.

Similar findings were obtained in studies by Rabindran et al,Saha et al,BekenS et al,Chawla et al.

- Correlation between LDH at 72 hours and severity of HIE

Our study found that there was a significant rise in LDH at 72 hours withincrease in severity of HIE.

Similar findings were obtained in studies by Rabindran et al,Patra et al, Muniraman et al, Beken S et al.

- Correlation between CKMB at 8 hours and outcome of HIE

Our study found that there was a significant rise in CKMB at 8 hours withworse outcome of HIE.

Similar findings were obtained in studies by Rabindran et al, Nakajima et al.

- Correlation between CKMB at 24 hours and outcome of HIE

Our study found that there was a significant rise in CKMB at 24 hours with worse outcome of HIE.

Similar findings were obtained in studies by Rabindran et al, Nakajima et al.

- Correlation between LDH at 72 hours and outcome of HIE

Our study found that there was greater rise in LDH at 72 hours with worseoutcome of HIE but this correlation did not have a significant p - value.

Similar findings were obtained in studies by Patra et al.

- Correlation between CKMB at 8 hours and need for intensive procedures

Our study found that there was a significant rise in CKMB at 8 hours withincrease in the need for intensive procedures. Similar findings were obtained in studies by Nakajima et al, Muniraman et al.

- Correlation between CKMB at 24 hours and need for intensive procedures

Our study found that there was a significant rise in CKMB at 24 hours withincrease in

the need for intensive procedures. Similar findings were obtained in studies by Nakajima et al, Muniraman et al.

➢ Correlation between LDH at 72 hours and need for intensive procedures

Our study found that there was a significant rise in LDH at 72 hours withincrease in the need for intensive procedures. Similar findings were obtained in studies by Nakajima et al, Patra et al,Muniraman et al.

SUMMARY

- In our prospective observational study we studied 55 neonates with perinatal 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 neurodevelopmental 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 neurodevelopmental disabilities.

CONCLUSION

- CKMB levels at 8 hours, 24 hours and LDH levels at 72 hours afterbirth 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. hus, t 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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