CERTIFICATE

Certified that this dissertation entitled "**A STUDY TO EVALUATE THE SIGNIFICANCE OF SERUM CREATINE KINASE MUSCLE BRAIN FRACTION (CK-MB) AND LACTATE DEHYDROGENASE (LDH) IN NEONATES WITH BIRTH ASPHYXIA"** is a bonafide work done by **Dr.S.KANIMOZHI** Postgraduate Student of Pediatric Medicine, Government Mohan Kumaramangalam Medical College, Salem, Tamil Nadu, during the academic year 2012-2015.

Dr.D. Sampath Kumar, MD., DCH.,
Associate Professor, Department of Paediatrics,
Government Mohan Kumaramangalam
Medical College, Salem

ASSOCIATE PROFESSOR
Pediatric Department.
Govt. Mohan Kumaramangalam
Medical College Hospital.
SALEM.

Certified that this dissertation entitled **"A STUDY TO EVALUATE THE SIGNIFICANCE OF SERUM CREATINE KINASE MUSCLE BRAIN FRACTION (CK-MB) AND LACTATE DEHYDROGENASE (LDH) IN NEONATES WITH BIRTH ASPHYXIA"** is a bonafide work done by **Dr.S.KANIMOZHI** Postgraduate Student of Pediatric Medicine, Government Mohan Kumaramangalam Medical College, Salem, Tamil Nadu, during the academic year 2012-2015.

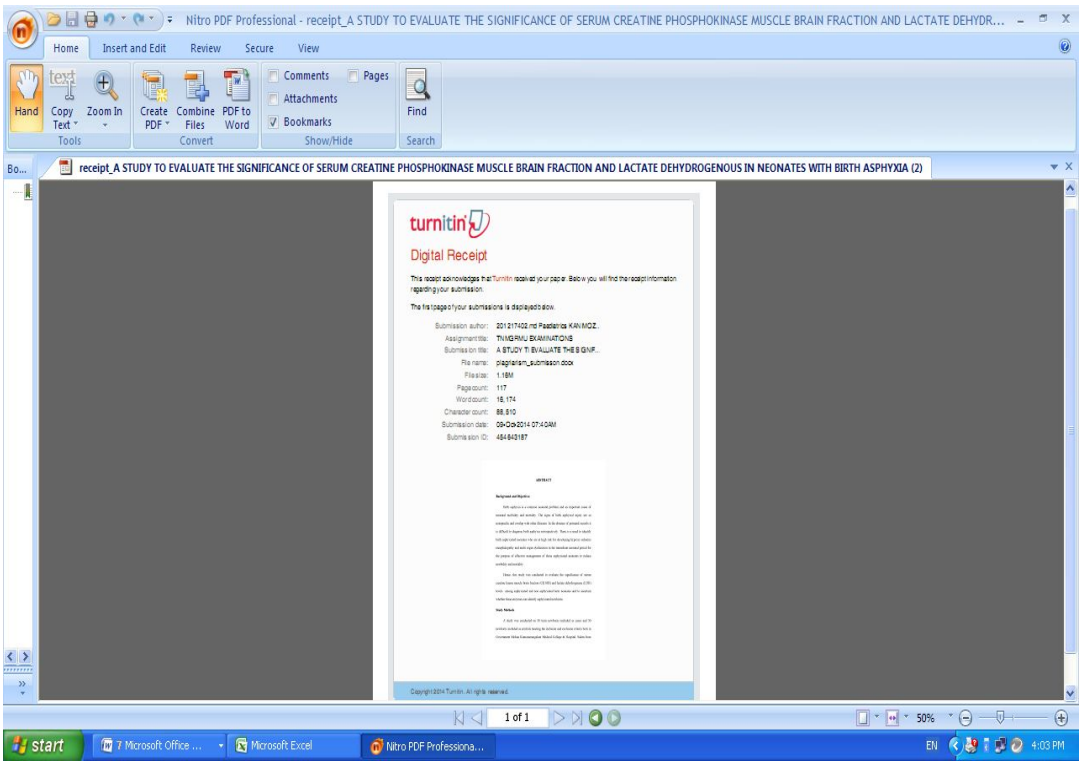
Dr.T.S. SUNDARARAJAN, M.D., D.C.H.,
Professor & HOD
Department of Paediatric Medicine,
Govt. Mohan Kumaramangalam Medical College,
Salem.

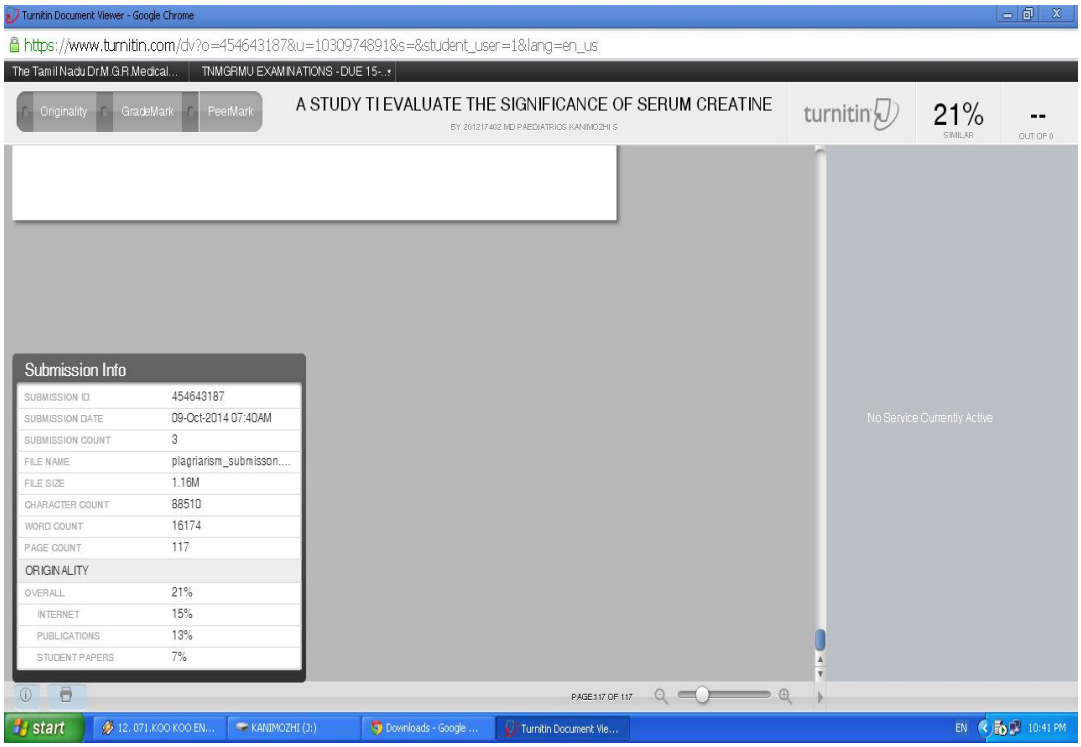
Professor and Head
Department of Pediatrics
Govt., Mohan Kumaramangalam
Medical College & Hospital
SALEM - 636 001

Certified that this dissertation entitled "**A STUDY TO EVALUATE THE SIGNIFICANCE OF SERUM CREATINE KINASE MUSCLE BRAIN FRACTION (CK-MB) AND LACTATE DEHYDROGENASE (LDH) IN NEONATES WITH BIRTH ASPHYXIA**" is a bonafide work done by **Dr.S.KANIMOZHI** Postgraduate Student of Pediatric Medicine, Government Mohan Kumaramangalam Medical College, Salem, Tamil Nadu, during the academic year 2012-2015.

17-10-14

Dr.N.MOHAN, M.S., FICS., FAIS.,
DEAN
Govt. Mohan Kumaramangalam
Medical College, Salem


Nitro PDF Professional - receipt_A STUDY TO EVALUATE THE SIGNIFICANCE OF SERUM CREATINE PHOSPHOKINASE MUSCLE BRAIN FRACTION AND LACTATE DEHYDR...
Home
Insert and Edit
Review
Secure
View
Hand
Copy Text
Zoom In
Create PDF
Combine Files
PDF to Word
Comments
Pages
Attachments
Bookmarks
Find
Tools
Convert
Show/Hide
Search
receipt_A STUDY TO EVALUATE THE SIGNIFICANCE OF SERUM CREATINE PHOSPHOKINASE MUSCLE BRAIN FRACTION AND LACTATE DEHYDROGENOUS IN NEONATES WITH BIRTH ASPHYXIA (2)
turnitin
Digital Receipt
This receipt acknowledges that Turnitin received your paper. Below you will find the receipt information regarding your submission.
The first page of your submissions is displayed below.
Submission author: 201217402 md Paediatrics KAN MOZ..
Assignment title: TNMGRMU EXAMINATIONS
Submission title: A STUDY TI EVALUATE THE B IGNIF...
File name: plagiarism_submisson.docx
File size: 1.18M
Page count: 117
Word count: 18,174
Character count: 88,810
Submission date: 09-Oct-2014 07:40AM
Submission ID: 464643187
ABSTRACT
Copyright 2014 Turnitin. All rights reserved.
1 of 1
50%
start
7 Microsoft Office ...
Microsoft Excel
Nitro PDF Professiona...
EN
4:03 PM


Turnitin Document Viewer - Google Chrome
https://www.turnitin.com/dv?o=454643187&u=1030974891&s=&student_user=1&lang=en_us
The Tamil Nadu Dr.M.G.R.Medical...
TNMGRMU EXAMINATIONS - DUE 15-...
Originality
GradeMark
PeerMark
A STUDY TI EVALUATE THE SIGNIFICANCE OF SERUM CREATINE
BY 201217402.MD PAEDIATRICS KANIMOZHI S
turnitin
21%
SIMILAR
--
OUT OF 0
Submission Info
SUBMISSION ID 454643187
SUBMISSION DATE 09-Oct-2014 07:40AM
SUBMISSION COUNT 3
FILE NAME plagriarism_submisson....
FILE SIZE 1.16M
CHARACTER COUNT 88510
WORD COUNT 16174
PAGE COUNT 117
ORIGINALITY
OVERALL 21%
INTERNET 15%
PUBLICATIONS 13%
STUDENT PAPERS 7%
No Service Currently Active
PAGE 117 OF 117
start
12. 071.KOO KOO EN...
KANIMOZHI (J:)
Downloads - Google ...
Turnitin Document Vie...
EN
10:41 PM

ABSTRACT

Background and Objectives

Birth asphyxia is a common neonatal problem and an important cause of neonatal morbidity and mortality. The signs of birth asphyxial injury are so nonspecific and overlap with other illnesses. In the absence of perinatal records it is difficult to diagnose birth asphyxia retrospectively. There is a need to identify birth asphyxiated neonates who are at high risk for developing hypoxic ischemic encephalopathy and multi organ dysfunction in the immediate neonatal period for the purpose of effective management of those asphyxiated neonates to reduce mortality and morbidity

Hence, this study was conducted to evaluate the significance of serum creatine kinase muscle brain fraction (CK-MB) and lactate dehydrogenase (LDH) levels among asphyxiated and non asphyxiated term neonates and to ascertain whether these enzymes can identify asphyxiated newborns.

Study Methods

A study was conducted on 50 term newborns included as cases and 50 newborns included as controls meeting the inclusion and exclusion criteria born in Government Mohan Kumaramangalam Medical College & Hospital, Salem from the period of June 2013 to may 2014. Cases are asphyxiated neonates and controls are non – asphyxiated neonates. The blood samples for CK-MB and LDH was drawn at 8±2 hours and 72±2 hours of age respectively and sent for analysis. A serum level of CK-MB > 92.6U/L at 8 hours and LDH >580 U/L at 72 hours was taken as the cut off value. The sensitivity, specificity, predictive value (positive and negative) was calculated for creatine kinase muscle brain fraction and Lactate dehydrogenase.

Results

The serum CK-MB value of >92.6U/L has 52% sensitivity with a specificity of 100%. CK-MB has a positive predictive value of 100 % with a negative predictive value of 58.14%. The cut off value of LDH was >580 U/L has 84% sensitivity with a specificity of 100%. Positive predictive value of LDH was 100% with a negative predictive value of 68.94%. LDH is having more diagnostic value than CK-MB with more

Area under ROC (receiving operating characteristic) value when compared to CK-MB.

Interpretation and Conclusion

From the study 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. CK-MB and LDH are the two biochemical markers useful in correlates with severity of HIE.

Keywords

Hypoxic Ischemic Encephalopathy, Creatine kinase muscle-brain fraction, lactate dehydrogenase, perinatal asphyxia, newborn

INTRODUCTION

Birth asphyxia is a common neonatal problem and contributes to significant morbidity and mortality. Birth asphyxia is an insult to the neonate due to lack of perfusion of vital organs of the body. In India, birth asphyxia contributes approximately 28.8% of neonatal death, a study conducted by NNPD (National Neonatal Perinatal Database) for the year 2002-2003[1].

Birth asphyxia is accounts for approximately 23% of the 4 million neonatal deaths and 26% of the 3.2 million stillbirths each year[2]. Approximately, 1 million children who survive birth asphyxia develop neuro developmental morbidities like cerebral palsy, learning disabilities, mental retardation. Mortality due to birth asphyxia in India is 2, 50,000 to 3, 50,000 each year[1]. Death usually occurs within first three days of life. Antepartum and intrapartum asphyxia contributes to 3, 00,000 to 4, 00,000 stillbirths[1]. In India 8.4% babies born with birth asphyxia with low apgar score less than 7 and among them 1.4% suffer from Hypoxic Ischemic Encephalopathy (HIE) and its sequelae[1]. Anticipation, early diagnosis and treatment are the important factors which alter the outcome of birth asphyxia. The signs of birth asphyxial injury are overlap with other illnesses. The babies born with asphyxia are brought late to health

care facility when the diagnosis may be indistinguishable from other illnesses. Without perinatal records to make a diagnosis of birth asphyxia is difficult.

Birth asphyxia is associated with multi organ injury with adverse neurological outcomes, but management of asphyxia is still focuses on supportive care. So, if adverse effects of birth asphyxia are considered, there is need for identification of infants who will be at high risk for developing adverse neurological outcome such as hypoxic ischemic encephalopathy and early neonatal death duo to birth asphyxia is important. There is a variety of markers have been used to identify neonates born with birth asphyxia including fetal heart rate monitoring , apgar score at 1 and 5 minutes, pH of umbilical cord blood at birth , electroencephalograms (EEG), magnetic resonance imaging(MRI), computed tomography (CT) and Doppler flow studies. The current problem is the inability to precisely differentiate true positive birth asphyxiated or compromised neonate from false positive newborns. So far several studies have been conducted to evaluate better markers to differentiate an asphyxiated from non asphyxiated newborns.

Birth asphyxia may affect all major body organs and these complications of birth asphyxia are very fatal. In a newborn with birth

asphyxia the percentage of multi organ involvement such as renal, neurological, cardiac, and lung dysfunction occurs in 50%, 28%, 25% and 23% of cases respectively[3]. Asphyxiated newborn with multi organ damage could manifest with seizures, encephalopathy, renal failure, respiratory distress, feeding intolerance and cyanosis. These signs and symptoms manifest as a single system disorder or occur in combination. The outcome of asphyxiated neonate is determined by extent of multi-organ damage and either the newborn succumbs as a consequence of organ damage or recovers completely. There are no long term sequelae associated with these organ dysfunction. Central nervous system dysfunction associated with birth asphyxia is referred as Hypoxic Ischemic Encephalopathy.

Transient myocardial ischemia (TMI) with myocardial dysfunction may occur in any neonate with birth asphyxia. An elevated levels of serum creatine kinase muscle-brain fraction (CK-MB) or serum cardiac enzymes like troponin T (cTnT) level may be useful in determining the presence of myocardial damage. An elevation of serum CK-MB fraction of >5% to 10% may indicate myocardial injury [4.]

Leakage of intracellular enzymes are useful in signalling multi organ damage. Serum aspartate aminotransferase (AST), alanine

aminotransferase (ALT) and lactate dehydrogenase (LDH) are useful in signaling multi organ damage is seen together with HIE after birth asphyxia[5-7].

The aim of the study was to evaluate the significance of serum levels of creatine kinase muscle brain fraction (CK-MB) and lactate dehydrogenase (LDH) among asphyxiated and non asphyxiated term newborns and to ascertain whether these enzymes can identify asphyxiated newborns and whether to predict the severity of birth asphyxia.

AIM OF THE STUDY

1. To evaluate the serum CK-MB and LDH level among asphyxiated and Non-asphyxiated term neonates.
2. To ascertain whether these enzymes can distinguish an asphyxiated from a non asphyxiated term neonate.
3. To ascertain whether these enzymes can be used to predict the severity of birth asphyxia.

REVIEW OF LITERATURE

HISTORICAL REVIEW

The examination of birth asphyxia from a historical perspective presents several intriguing problems. First, the definition of birth asphyxia was not well defined. Physician, biochemists and pathologist all use the asphyxia as pharse, but there is no universal definition. Dr. Eastman of Hopkins called asphyxia “an infelicity of etymology”. The asphyxia is a Greek word which means as without pulse[8].

A second problem seems to be that within each speciality studying asphyxia, once a definition is established the exceptions are enormous. According to the pathologist “asphyxic” lesion may occur without clinical or biochemical history of asphyxia. According to physiology textbook the definition of asphyxia includes hypoxia and hypercarbia. Alternatively, biochemical evidence of asphyxia is present in large number of children, are clinically completely normal.

The University of Pittsburg published a study paper describing the effect of birth asphyxia on children. The results were based on study conducted on 38,405 consecutive deliveries. The study result showed the relationship between prematurity and asphyxia. They also reported the positive relationship between gestational age and survivors of the

newborn. According to the report the incidence and severity of asphyxia were not related to gestational age[9].

In the study the only criteria are used to diagnose the asphyxia is neonate who requires positive pressure ventilation for more than one minute before sustained respiration has occurred. The study did not mention the specific etiology that lead to the absence of voluntary respiratory effort, and not mentioned any reference made to blood biochemistry.

In 1861 Dr.William Little presented a paper defining a causal relationship between the central nervous damage and abnormal parturition[10]. Dr.Little's study stated that, circulatory failure was an important cause of the central nervous system pathology. The expressions in vogue to describe asphyxia in little's time included "asphyxia neonatorum and suspended animation" a term not different from the Pittsburgh author's. He compared the newborn with asphyxia to drowning victims. Most interesting from the historical perspective is his observation that "the majority of stillborn infants are saved by attendant accoucheur recover unharmed from that condition."

In reviewing the history of asphyxia, one name in perinatal medicine which should be central is DR.N.J.Eastman. Dr.Eastman's work

began in the early 1930's and was based on principles derived from great physiologist. The physiologists were working in the area of respiration at that time[11].

According to Dr.Eastman the definition of asphyxia" is inability of the newborn to breath and apnea associated with oxygen deficiency during labour". Dr.Eastman was interested in the factors which was responsible for the initiation of respiration at birth whether hypoxia or hypercarbia. He thought that only by understanding the normal initiation of human respiration and biochemistry involved at the time of initiation of respiration, then only we know the abnormalities associated with abnormal respiration, i.e., asphyxia. He published series of five articles between 1931 to 1936. His next paper showed the maternal and fetal lactate relationship. He measured lactate level in cord blood of 24 neonates, 7 neonates had birth asphyxia. His paper showed lactate level was a measure of mild oxygen deficiency. He also stated that in the presence of adequate fetal oxygen there will be absence of hyperlactatemia[12].

A German investigator, Heinbicken in 1929 demonstrated that acidic products from anoxemia could cause cellular damage. The summary of these two paper and their clinical application is compiled by

Dr Eastman's third paper on the subject. He quoted the study of Kreiselman and Kane in 1930 showing increased carbon dioxide level in the asphyxiated adult patients. He measured pH and carbon dioxide levels in maternal and fetal blood. Lastly, he described that asphyxia accompanies acidosis.

Scimidt in 1928 stated that after prolonged hypoxia and hypercarbia, the respiratory center no longer could utilize oxygen and respiratory depression ensued. In 1910 Mathison described the effect of asphyxia on reducing cardiac output.

In 1953 Dr.Apgar in published a paper and she was obviously disturbed by the lack of specificity in resuscitation. In her paper stated that the lack of systemic evaluation of newborns which was the limitation of the evaluation of resuscitation methodology. She chose criteria to obviate the need for intervention during the resuscitation efforts. She felt that her criteria could be delineated without compromising care. She made a correlation of the score with a variety of birth variables include perinatal mortality and type of anaesthesia. She showed the inverse relationship of the score to the need for active resuscitation[13]. Dr.apgar extended her work with several of her associates, notably by Dr.L.S.James. James and his coworkers converted the Apgar criteria into

acid base biochemical correlates. Since one of the limitation of the historical observation in asphyxia is the lack of coordination among pathological and biochemical and clinical phenomena, but this less dramatic work is of critical importance[14].

Dr.William Windle used a subhuman primate as a modal for his study and many of the pathophysiology of the sequelae is clearer. He made a most important correlation between pathological findings, biochemical values[15] and clinical state.

Dr. Mayers study described the effect of hypoxia on brain. Hypoxia leading to cellular damage which likely to cause brain damage. As the cellular swelling occurs with membrane injury which leads to secondary loss of membrane integrity. The loss of membrane integrity leads to ischemia which further decreasing oxygen damage. Since cardiac muscle is affected simultaneously resulting in reduced cardiac output leading to hypoperfusion[16]. Since the goal should be prevention of asphyxia, based on the understanding of the underlying involved mechanisms, a various markers have been examined to identify birth asphyxia including fetal heart rate monitoring, intrapartum fetal scalp pH monitoring, low apgar scores, pH of the umbilical cord blood , EEG, CT, MRI scans and doppler flow studies.

Based on historical review the current problem is inability to precisely differentiate the true positive asphyxiated or compromised newborn from false positive newborns. Several studies have been conducted to evaluate the better markers that help to distinguish an asphyxiated from non asphyxiated newborns.

In 1985 a study was conducted by **primhak et al** stated that serum peak level of CK-MB seen in both normal and asphyxiated neonate at 8 hours and fell by 72 hours. Higher Absolute and percentage CK-MB levels were seen in asphyxiated babies[17].

A study was conducted by **sanchez nava et al** in 1990 and his study showed that birth asphyxiated neonates the biochemical markers such as AST, ALT and LDH were raised[18].

In 1991, a study conducted by **Omokhodion SI et al** showed the serum creatine kinase (CK-MB) levels in 23 perinatally asphyxiated newborns and 12 healthy controls during first 100 hours of life. The asphyxiated newborns had significantly elevated mean CK and absolute CK-MB but no fractional CK-MB activities. Peak mean CK and CK-MB values were 789.17±220 U/L with P value less than 0.01 and 16.36 ±3.0 U/L with p value less than 0.001 respectively at 6 to 8 hours of post natal

period. The healthy controls showed a steady decline in the activities of these enzymes from birth[19].

In 1995 **Fonseca E et al** showed that antepartum fetal distress is associated with release of biochemical markers such as CK-BB and CK-MB. Fonseca E et al study stated that biochemical markers CK-BB indicate brain damage and CK-MB indicate myocardial damage[20].

In 1996 **Lackmann et al** conducted a study and concluded that asphyxiated neonates have significantly higher values of AST, LDH and hydroxybutyrate .These higher value of the biochemical markers compared to neonates only with RDS. The presence of RDS among asphyxiated neonates did not alter the enzyme level[21].

In 1999 **Barberi et al** reported that the various biochemical markers such as CK, CK-MB, CK-MB/CK ratio and LDH were all increased in an asphyxiated group. In a babies with respiratory distress only CK-MB, CK-MB/CK ratio were abnormal[22].

In 2000 **Karunatilka DH et al** conducted a study in Sri Lanka to evaluate the usefulness of CK. He evaluated the usefulness of CK-MB alone or in combination with LDH in identifying high risk newborns developing HIE or major handicap following birth asphyxia. Their study showed in birth asphyxia both the CK and LDH values are raised. The

markedly increased values are noted among those who developed HIE. Their study showed the correlation between raised CK levels with long term outcome. The serum CK values above 2860 IU/L should be monitored for both immediate and long term outcome[23].

Boo NY et al conducted a study in 2005 stated that asphyxiated babies showed significantly higher concentrations of cTnT and CK-MB than controls[24]. Asphyxiated newborns died of cardiac dysfunction or developed cardiac dysfunction had significantly higher serum cTnT concentrations compared to CK-MB.

Reddy S et al conducted a study in 2008 showed the sensitivity and specificity of CK-MB and LDH. In birth asphyxia LDH had 100% sensitivity, while CK-MB had 100% specificity. They also concluded that 72 hours of life LDH is the most accurate at differentiating asphyxiated from non asphyxiated symptomatic newborns[25]. The study stated that LDH level could be used at 3 days of age to make a retrospective diagnosis of birth asphyxia.

In 2008, a study conducted by **Rajakumar PS et al**, when compared to controls the cardiac enzymes cTnT and CK-MB levels were significantly raised in cases. The study showed that mean CK-MB levels among cases were 121±77.4 IU/L and controls were 28.8±20.2 IU/L. The

sensitivity and specificity of CKMB level were 75.7% and 56.5% respectively[26].

In 2008, a study was conducted by **Karlsson M et al**, on evaluation of organ damage in birth asphyxia[27]. He concluded that in asphyxiated babies with HIE and infants with signs of fetal distress during birth a cut off level of 1049 U/L for LDH was the most suitable predictor of mild, moderate and severe HIE. LDH had a sensitivity of 100% and specificity of 97%.

Birth Asphyxia

Birth asphyxia is the common and preventable cause of cerebral injury occurring in the newborn period. Although birth asphyxia is a commonly made diagnosis the definition of birth asphyxia is controversial. Asphyxia is an abnormal process, at a pathophysiological level simultaneous combination of hypoxia and hypoperfusion which leads to tissue acidosis.

National neonatology forum of India has suggested that asphyxia should be diagnosed when baby has gasping at birth and inadequate respiration or no respiration at 1 minute. National neonatology forum India definition is simple, easy to use and apply.

According to WHO definition, birth asphyxia defined as “the failure to initiate and sustain breathing at birth”[28].

American academy of pediatrics defines birth asphyxia as[29]

- Cord umbilical artery pH of <7.0 with base deficit of >10 meq/L.
- Neonatal neurological manifestation suggestive of hypoxic ischemic encephalopathy.
- Evidence of multisystem organ dysfunction (cardiovascular system, renal system, gastrointestinal system, pulmonary system).

This definition showed a good correlation with neonatal mortality and the subsequent chances of occurrence of cerebral palsy.

The ICD-10 definition of birth asphyxia is based on the Apgar scoring system. The ICD-10 definition of birth asphyxia is dependent on the apgar score at 1 minute of age. An Apgar score at 1 min of 0-3 defines severe birth asphyxia and apgar score of 4-7 defines moderate birth asphyxia[30].

The NNPD 2000 defined moderate asphyxia as slow gasping breathing or an apgar score of 4-6. Severe asphyxia defined as no breathing or an Apgar score of 0-3 at one minute of life[31].

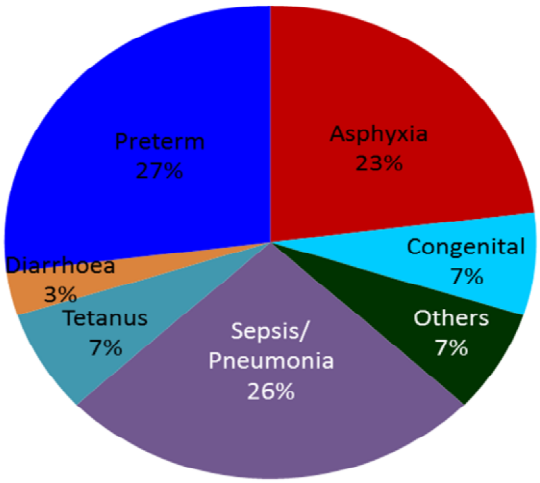
Incidence of Neonatal Death

Globally, 130 million babies are born every year and of these 4 millions die during the neonatal period i.e. during first four weeks of life. A similar number of babies are still born. These accounts for 8 million perinatal deaths per year.15 lives are lost every minute. 75% of neonatal deaths occur in first week of life. Approximately 25% of neonatal death occur during first 24 hours.The risk of mortality is 30 fold higher during neonatal period than the post neonatal period. Almost 98% of neonatal deaths occur in resource limited countries. India accounts for highest number of annual birth approximately 27 million and highest number of neonatal deaths approximately 1.2 million.Neonatal death accounts for two-third of all infant deaths and 40% of under 5 child deaths. The millennium development goal 4 (reducing under 5 mortality by two–thirds) cannot be achieved without substantial reduction in neonatal mortality[32].

According to WHO 2000[32] estimates the direct causes of neonatal deaths include

- Preterm birth - 27%
- Severe infections - 36%
- Birth asphyxia - 23%
- Congenital malformation - 7%

Figure: 1. Estimated distribution of direct causes of global neonatal deaths[32]



During intrapartum period each normal fetus experiences an episode of hypoxemia, hypercapnea and mixed acidosis. This occurs as a result of impaired blood flow in the uterus during labour and no signs of neurological dysfunction occur following this mild asphyxial episode. Birth asphyxia is the simultaneous combination of both hypoxia and hypoperfusion at a pathophysiological level. This may cause impaired gas

exchange that lead to tissue acidosis. There is variability in 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 suggested that the term birth asphyxia should no longer be used[33]. Since there is simultaneous occurrence of hypoxia and ischemia the term hypoxic ischemic insult is now preferred. Hypoxic ischemic insult can no doubt lead to severe brain injury but a major problem regarding the term is in those children who develop long term neurodevelopmental disability such as cerebral palsy. In these children there is an false assumption that they were injured during labour and delivery. As a result that obstetricians and midwives are targeted as the person responsible for neurological sequelae[34].

Newer terms include ‘birth depression’, which is a descriptive term to indicate a newborn with poor apgar but without judgement on etiology. The use of word ‘perinatal’ rather than ‘birth’ supports the pathological processes that may begin many hours before birth and continue for many hours afterwards. There are many causes and the clinical manifestations may vary. Infants with mild birth asphyxia show no neurological injury. However newborns with severe birth asphyxia may be fatal in utero, or

immediately after birth and survivors of birth asphyxia show extensive neurological sequelae, with or without cognitive defects[30].

Some of the terms are used in evaluating a term infant at risk for brain injury in the perinatal period are as follows[35]:

1. Perinatal hypoxia, ischemia and asphyxia

These pathophysiological term defined lack of oxygen, blood flow and gas exchange to the newborn respectively.

2. Perinatal Depression

Perinatal depression is a descriptive, clinical term that pertains to the condition of the newborn in the immediate post natal period (i.e., in the first hour after birth).The clinical features includes muscle hypotonia, depressed mental status and disturbances in cardiovascular function and spontaneous respiration[35].

3. Neonatal Encephalopathy

It is a clinical term used to describe an abnormal neurobehavioral state that consists of a depressed level of consciousness with abnormalities in muscular tone, and other signs of brain dysfunction. It begins within the first postnatal life and newborn presents with depressed primitive reflexes and presence of brain stem reflexes, seizure like

activity, hypoventilation or apnea[35]. It does not imply a specific etiology, nor does it imply irreversible neurologic injury. It may be caused by maternal medications or hypoglycemia.

4. Hypoxic – Ischemic Brain Injury

It is a neuropathological term attributable to perinatal hypoxia and/ or ischemia as evidenced by biochemical abnormalities (such as increase in the serum creatine kinase brain bound (CK-BB), abnormal neuroimaging (cranial ultrasonography, CT, MRI) and electro encephalogram abnormalities (EEG) or postmortem abnormalities[35].

5. Hypoxic – Ischemic Encephalopathy (HIE)

HIE defined as encephalopathy with objective data to support a hypoxic ischemia as the underlying cause for the encephalopathy[35].

Magnitude of the problem

Birth asphyxia is one of the leading cause of neonatal morbidity and mortality in worldwide. The incidence of perinatal asphyxia is more in developing countries like India. The incidence of birth asphyxia is approximately 1% to 1.5% of live births in the western Hemisphere.The incidence of birth asphyxia is inversely related to gestational age and birth weight. It occurs in 0.5% of live born infants of more than 36 weeks

of gestational age. It accounts for 20% of perinatal deaths[4] and if stillborn are included the incidence will be 50%.

Infant of diabetic mothers and toxemia of pregnancy have a higher incidence of birth asphyxia. A higher incidence of birth asphyxia noted in newborns with IUGR, breech presentation and abnormal presentation and postdated infants. In India 8.4% of babies are born with 1 minute apgar score less than 7. Out of these 1.4% were suffer from HIE[1].

Assessment of Fetal Well – Being

Many assessments were made attempted to predict fetal well being during labour and following delivery. These include meconium stained amniotic fluid, electronic fetal heart rate monitoring by cardiotocograph (CTG), Apgar score and the assessment of fetal acid base balance.

1. Meconium Staining Amniotic Fluid

Thick meconium staining or heavy meconium staining is a marker of prolonged or severe asphyxial episodes. Meconium staining is seen in 15% of all labours and 11% of term pregnancies where there is no evidence of asphyxia other than meconium stained amniotic fluid. [36] However, only 0.4% of term babies with meconium stained amniotic fluid during labour subsequently developed cerebral palsy[37].

Richey et al[38] in his study showed that there was no correlation between the meconium stained amniotic fluid and markers of acute asphyxia such as umbilical artery P^H, serum lactate and hypoxanthine level. This sign is poor predictor of adverse outcome and in one study more than half of infants develop early neonatal seizures (a possible indicator of intrapartum asphyxia) showed no evidence of meconium staining. If cerebral palsy is taken as the endpoint of a major asphyxial event in the perinatal period, then 99.6% of normal birth weight babies with meconium staining had no evidence of this condition[37].

2. Electronic Fetal Monitoring (EFM)

Continuous electronic fetal monitoring is commonly used despite it has not been shown to reduce perinatal mortality or birth asphyxia but has increased the incidence of operative delivery[4]. When used, these monitors simultaneously record fetal heart rate and uterine activity for ongoing evaluation[4].

Parameters that are used for fetal monitoring include the following[39];

1. Normal Baseline fetal heart rate is between 110 and 160 bpm (beats per minute). The baseline heart beat apparent for a minimum 2 minutes in any 10 minute segment .The baseline heart rate does not include episodic changes, period of marked FHR variability and baseline heart beat that differ by more than 25 bpm. Baseline bradycardia, defined as FHR <110bpm. Fetal bradycardia may result from congenital heart block associated with congenital malformation or maternal systemic lupus erythematous. Baseline tachycardia, defined as an FHR > 160 bpm, may result from a fetal dysrhythmia, hyperthyroidism, maternal fever or chorioamnionitis.

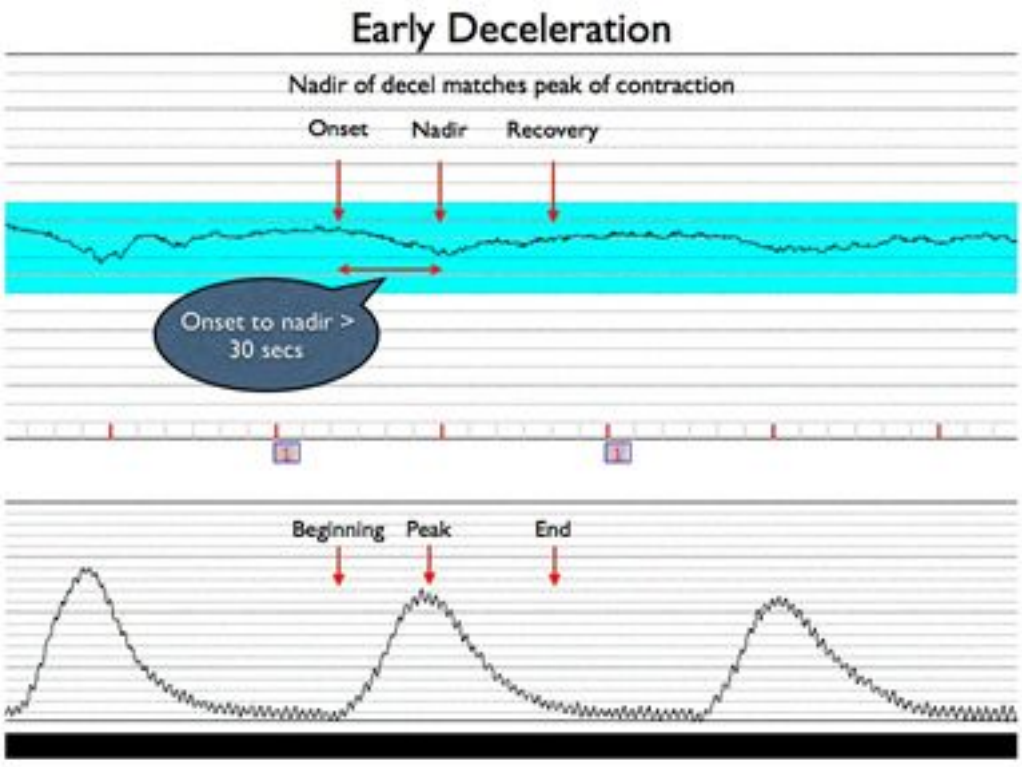
2. Beat to beat variability is recorded from a calculation of each RR interval. The autonomic nervous system of healthy, awake fetus constantly varies the heart rate from the beat to beat by approximately 5 to 25 beats per minute. Reduced beat to beat variability may result from depression of the fetal central nervous system due to fetal immaturity, hypoxia, fetal sleep, or specific maternal medications such as narcotics sedatives, beta blockers, and intravenous magnesium sulfate[39].

3. Accelerations of the FHR are reassuring during a non stress test (NST).

4. Decelerations of the FHR may be benign or indicative fetal compromise[39]depending on their characteristic shape and timing in relation to uterine contractions.

a. Early decelerations are symmetric in shape and usually accompany with beat to beat variability. These decelerations are commonly seen in active labour when the fetal head is compressed in the pelvis resulting in a parasympathetic effect.

Figure: 2. Shows Fetal Heart Rate Tracing[40] – Early Deceleration



b. Late decelerations are decreases in the FHR in association with uterine contractions. Fetal heart rate begins to decelerate 15-30 seconds after the onset of uterine contraction reaches the nadir after the peak of the contraction and does not reaches the baseline even after the cessation of uterine contraction. A fall in the heart rate of 10 to 20 bpm below the baseline is significant. Late decelerations are the result of uteroplacental insufficiency and fetal hypoxia. As the uteroplacental insufficiency worsens,

i. Loss of beat to beat variability

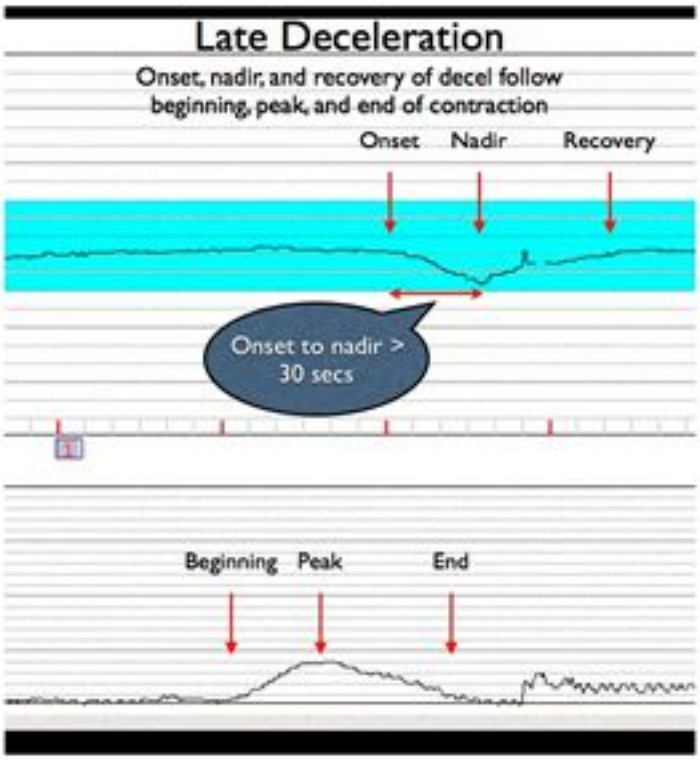
ii. The deceleration will last longer

iii. They will begin sooner following the onset of a contraction

iv. They will take longer to return to baseline

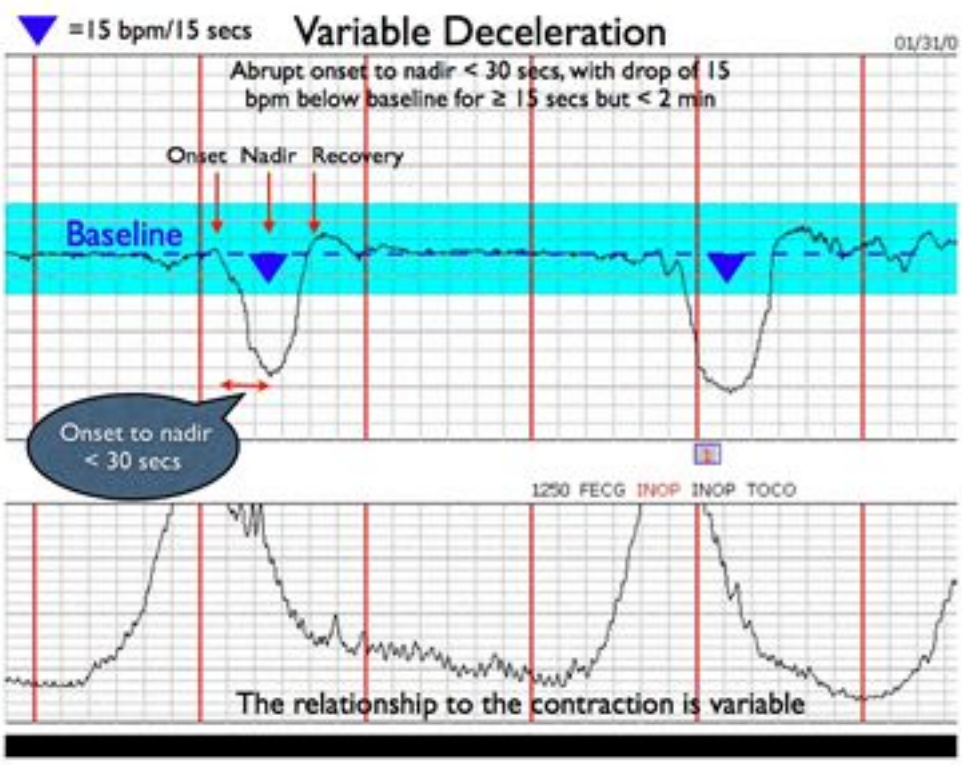
v. Repetitive late decelerations need immediate action.

Figure: 3. Shows Fetal Heart Rate Tracing[41] - Late Deceleration



c. Variable deceleration vary in their shape and in their timing relative to contractions. Usually they result from fetal umbilical cord compression. Variable decelerations are cause for concern if they are severe (down to a rate of 60bpm or lasting for 60 seconds or longer or both), associated with poor beat to beat variability or mixed with late decelerations.

Figure : 4. Shows Fetal Heart Rate Tracing[41] – Variable Deceleration



3. Fetal activity record

This is the simple method by which mother herself can check the health of her own baby. It involves counting the quickenings or number of movements made by the baby during third trimester of pregnancy. Mother is advised to count the number of fetal movements everyday starting in the morning until the total movements equals ten and is recorded in the chart. If mother feels less than ten movements per day for two consecutive days, she must report to the doctor on the following day. If mother does not appreciate any fetal movements in a day, she must contact the doctor immediately[42].

4. Non stress Test (NST)[43]

Non stress test is a reliable means of fetal evaluation. It is simple to perform and noninvasive test. This is simultaneous and the continuous recording of fetal heart rate and uterine contractions and movement of fetus provide useful information.

The criteria for "**Reactive" NST** are as follows[43]

i) Fetal heart rate between 110 and 160

ii) Normal beat to beat variability (5bpm)

iii) Two acceleration of heart beat by 15 or more for 15 seconds association with movements of the fetus in a 20 minute record period.

'Non Reactive Test (NST)'

A non reactive test fails to meet above criteria[43]

Inadequate test

If adequate fetal heart rate tracing cannot be obtained, the test is considered inadequate[43].

Statistics show that reactive test is reassuring, with the risk of fetal demise within the week following test approximately 3 in 1000.

A non reactive test should be repeated later the same day or is followed by another test of fetal well being

A persistently slow fetal heart rate without any variability to uterine contractions or fetal movements is indicative of fetal hypoxia[43].

5. Oxytocin challenge test

It is a useful test to assess the integrity of uteroplacental unit. Uterine contractions are induced with oxytocin and their effect on fetal heart rate is monitored.

6. Fetal biophysical profile (manning score or planning score) is the most accurate and non-invasive parameter and assessed by real time ultrasound[44].

Table : 1. Shows Fetal Biophysical Profile[44]

Biophysical variable	Normal (score 2)	Abnormal (score 0)
Posture	Flexed	Extended
Fetal breathing movements	At least one episode of FBM of atleast 30 sec duration in 30 min	2 or fewer body/limb movements in 30 minutes
Gross body movements	Atleast 3 discrete body /limb movements in 30 min	2 or fewer body /limb movements in 30 min
Reactive FHR	Atleast 2 episodes of FHR acceleration of 15 bpm of at least 15 sec duration in a period of 30 min	Less than 2 episodes of acceleration of >15 bpm in 30 min
Amniotic fluid volume	Atleast 1 pocket of AF measuring 1 cm or more in 2 perpendicular direction	No AF pocket or <1.0 cm in 2 perpendicular directions

The fetus is assessed ultrasonically for atleast 30 minute period.

7. A fetal scalp blood sample for blood gas analysis to confirm or dismiss suspicion of fetal hypoxia. An intrapartum scalp pH above 7.2 with a base deficit <6 mmol/L is normal. FHR accelerations in response to mechanical stimulation of the fetal scalp or to vibroacoustic stimulation are reassuring[44].

8. Doppler velocimetry studies

Doppler studies of uterine artery and umbilical artery are reliable to assess the adequacy of uteroplacental circulation of the fetus[44].

Etiology

In term infants, 90% of birth asphyxia occurs in the antepartum or intrapartum period. Birth asphyxia occurs as a result of impaired gaseous exchange across the placenta. Impaired gaseous exchange of placenta leads to the inadequate provision of oxygen (O_2) and removal of hydrogen (H^+) and carbon dioxide (CO_2) from the fetus. The remainder of these events occurs in the postpartum period. The remainder of the events occurs usually secondary to neurological, cardiovascular, pulmonary or renal abnormalities[4].

A. Various factors that increase the risk of birth asphyxia include the following[45];

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

B. Etiologies of hypoxia-ischemia include the following;

1. Maternal risk factors
    a. Acute or chronic hypertension, hypotension
    b. Infection including chorioamnionitis
    c. Hypoxia from pulmonary or cardiac disorders
    d. Maternal Diabetes, maternal vascular disease
    e. In utero exposure to cocaine.
2. Placental factors
    a. Abnormal placentation
    b. Abruptio placenta,
    c. Infarction and fibrosis.
3. Rupture of the uterus
4. Umbilical cord accident
    a. Cord prolapse
    b. Cord entanglement
    c. True knot
    d. Cord compression.
5. Umbilical vessels abnormalities
6. Fetal factors
    a. Fetal Anemia
    b. Infection

c. Cardiomyopathy

d. Hydrops fetalis, severe cardiac or circulatory insufficiency.

7. Neonatal factors

a. Cyanotic congenital heart disease

b. Persistent Pulmonary Hypertension of the Newborn (PPHN), Cardiomyopathy,

c. Neonatal cardiogenic and or septic shock.

Clinical Features

A baby with birth asphyxia may present with any symptoms in the form of respiratory distress, congestive cardiac failure, abdominal distention, poor feeding, seizures, lethargy, hypotonia, bleeding, shock, hypoglycemia[45].

Some or all of these clinical features may be found in newborn with other illnesses like sepsis, intraventricular hemorrhage, pneumonia and hyaline membrane disease. The most important of which is neonatal sepsis with multi organ involvement.

The following conditions are present with similar symptomatology as seen in birth asphyxia[45]

1. Respiratory distress: Sepsis, CCF, RDS, MAS, TTN, congenital pneumonia, congenital anomalies.
2. Lethargy: Hypoglycemia, sepsis, CNS malformations.

3. Temperature instability: Sepsis, dehydration, CNS disease.
4. Gastrointestinal disturbances: Sepsis, dehydration, congenital adrenal hyperplasia, hypokalemia.
5. Seizures: Sepsis with meningitis, intracranial hemorrhage, dyselectrolytemias, inborn error of metabolism.
6. Petechiae: Sepsis, immune thrombocytopenia, congenital leukemia.

In the absence of proper birth history and laboratory investigations it is very difficult to distinguish birth asphyxia from other illnesses.

Apgar Score

In 1952, **Dr. Virginia** Apgar devised a scoring system[13] to assess the newborn condition at birth. It was a rapid method of assessing the clinical status of the newborn at 1 minute of age. The scoring system is helpful for prompt intervention to establish breathing. In 1958, a second report was published. The second report scoring system provided a standardized assessment for newborn after delivery. The five components of the apgar score included the following: **A**ppearance, **p**ulse, **G**rimace, **A**ctivity, **R**ate. Each component is given a score of 0, 1, or 2. The score is reported at 1 and 5 minutes after birth[46].

Table: 2.Apgar Evaluation of Newborn Infants[46]

Sign	0	1	2
Heart rate	Absent	<100 bpm	> 100 bpm
Respiratory effort	Absent	Slow , Irregular	Good ,crying
Muscle tone	Limp	Some flexion of extremities	Active motion
Reflex irritability	No response	Grimace	Cough or sneeze
Color	Blue, pale	Body pink, extremities blue	All pink

Limitations of the Apgar Score

Apgar score has many imitations. The apgar score is an expression of the infant's physiological condition. Apgar score has a limited time frame and includes subjective components. In addition the biological disturbances occur must be significant before the score is affected.

The incidence of low apgar score is inversely related to birth weight. The low apgar score is limited in predicting neonatal morbidity or mortality. Apgar score alone inappropriate to establish the diagnosis of birth asphyxia. There is sufficient evidence suggest that there is poor correlation between apgar score, cord pH ,and future mental prognosis of asphyxiated newborns.However 10 minutes apgar score 3 or less or no spontaneous respiration at 10 minutes, the baby is likely to develop neuromotor disability during follow up.[47]

Apgar Score and Resuscitation

During resuscitation, the 5 minute apgar score, and particularly a change in the score 1 and 5 minutes is most useful index of the response to resuscitation. According to NRP guidelines if the apgar score is less than 7 at 5 minutes, the assessment should be repeated every 5 minutes upto 20 minutes. However, an apgar scoring system assigned during resuscitation is not equivalent to a score assigned to a spontaneously breathing neonates. There is no accepted standard for reporting an apgar score in newborns undergoing resuscitation. Many of the elements contributing to the score can be altered by resuscitation[48].

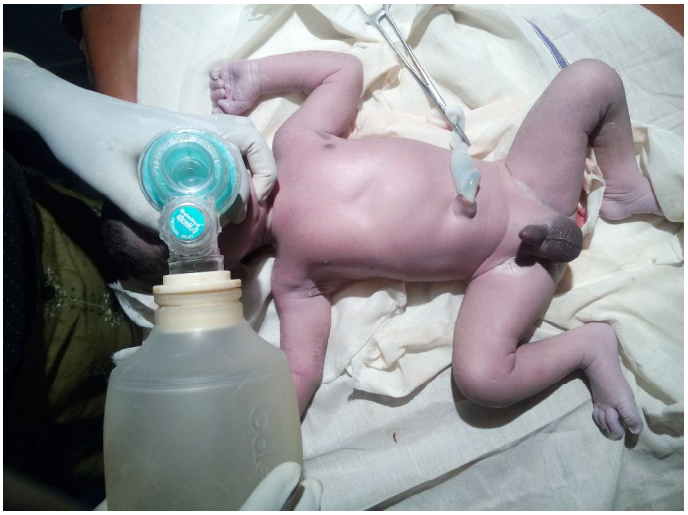


Figure: 5. Resuscitation of an Asphyxiated Baby

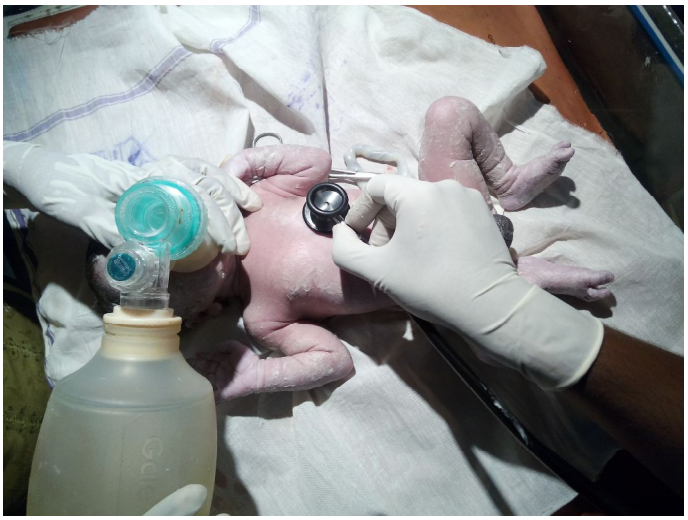


Figure: 6. Bag and Mask Ventilation

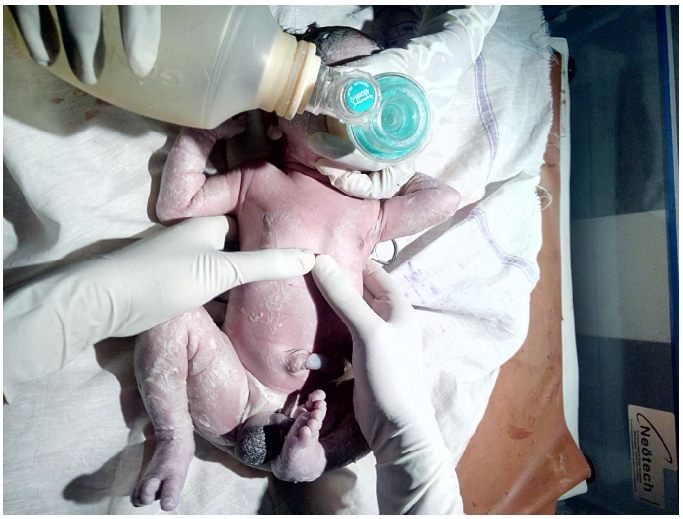


Figure: 7.
External Cardiac Massage

Prediction of Outcome

To predict the outcome of newborn born with asphyxia a low 1-minute Apgar score alone does not make any correlation with the infant's future outcome. A retrospective analysis was done and concluded that the 5-minute Apgar score is a valid predictor of neonatal mortality. But using 5 minute apgar score to predict long-term outcome was inappropriate.Another study stated that low Apgar scores at 5 minutes are associated with death or cerebral palsy. But the association between low apgar at 5 minute and neonatal morbidity and mortality was increased when both 1- and 5-minute scores were low[49].

An 5 minutes apgar score in term infants correlates poorly with neurological outcome in future. A 5 minute score of 0 to 3 was associated with a slightly increased risk of cerebral palsy when compared with higher scores. Conversely, 75% of children with cerebral palsy had normal 5 minute apgar scores. An Apgar score at 5 minute of 7 to 10 is considered normal. Apgar scores of 4, 5 and 6 are considered as intermediate and are not markers of increased risk of neurologic dysfunction. Such low apgar scores may be the result of physical immaturity, the congenital malformations, maternal medications, and other factors. Because of these other conditions, the low Apgar score

alone cannot be considered as evidence or a consequence of birth asphyxia[50].

Other factors need to be considered when defining intrapartum hypoxia-ischemia as a cause of cerebral palsy. The factors need to be considered are fetal heart rate monitoring including non reassuring NST and abnormalities in umbilical arterial blood gas analysis, neuroimaging studies, electroencephalography (EEG), pathological abnormalities of placenta, hematological studies, and multi organ dysfunction[48].

Factors affecting apgar score[51]

False – Positive apgar score

(Low apgar score, no fetal hypoxia or acidosis)

- Analgesics
- Narcotics
- Precipitous delivery
- Immaturity
- Acute cerebral trauma
- Congenital myopathy or neuropathy
- Spinal cord trauma
- Central nervous system anomaly & lung anomaly (diaphragmatic hernia)
- Airway obstruction (choanal atresia)
- Congenital pneumonia
- Hemorrhage - hypovolemia

False – Negative (acidosis present, normal apgar)

- High catecholamine levels
- Maternal acidosis
- Some full term infants

Table : 3. Incidence of Neonatal Death (in 132,228 Infants) Born at Term in Relation to Apgar Scores at 5 Minutes of Age[51]

5 min apgar score	No of live births	No of neonatal deaths (rate per 1,000 births)	Relative risk (95% CI)
0-3	86	21 (244)	1,460 (835-2,555)
4-6	561	5 (9)	53(20-140)
7-10	131,581	22 (0.2)	1

American college of obstetricians and gynecologist and American academy of pediatrics concluded the issue on apgar score and made the statements are following;

As some component of the apgar score are dependent on the physical maturity of the neonate. A healthy preterm newborn may have a low apgar score because of immaturity[52].

Correlation between the low apgar score with adverse neurological outcome increases when the score is 3 or less at 10, 15 and 20 minute.

But presence of low apgar score does not indicate or predict the adverse neurological outcome in future[52].

Need For Correct Diagnosis of Post Asphyxial Organ Injury

The need for correct diagnosis of birth asphyxia with many nonspecific clinical features is extremely important as the long term prognosis of perinatal asphyxiated and non asphyxiated newborns is totally different. Long term neurological sequelae of birth asphyxia are the following[53];

- Seizure disorder
- Impairment of cognitive function, learning disability, neuropsychological disturbances.
- Hearing impairment
- Visual impairment
- Mental retardation
- Cerebral palsy- spastic diplegia or spastic quadriplegia

For the above reasons regular follow up is necessary in asphyxiated than in non- asphyxiated newborns.

The low apgar score alone cannot establish birth asphyxia as the cause of cerebral palsy. A newborn who had an asphyxial injury close to

delivery that is severe enough to cause neurological injury should demonstrate all of the findings.

The clinical criteria to establish the acute neurological injury in the neonate was related to birth asphyxia proximate to delivery are[54]:

1. Metabolic acidemia determined by an umbilical cord arterial sample (pH<7.0)
2. Neonatal neurological manifestations e.g. hypotonia, seizures or coma
3. Apgar score of 0-3 for greater than 5 min
4. Multi system organ dysfunction: Cardiovascular, renal, gastrointestinal, pulmonary or hematological system

Pathophysiology of Hypoxia-Ischemia

When a cell is exposed to hypoxia or ischemia the outcome depends on the duration and degree of the insult. If the duration of the insult is brief the cellular injury may be reversible and if prolonged injury, the cell will be irreversibly injured and die. The two main pathological changes that occur during cell death are necrosis and apoptosis. Necrosis is seen after loss of blood supply to the cell and also if cell is exposed to toxins. Apoptosis is seen in under both physiological

and pathological conditions. The pathophysiological mechanism of cell death are presented in figures: 5 to 7

Figure : 8. Cellular and Biochemical Sites of Damage in Cell Injury[55]

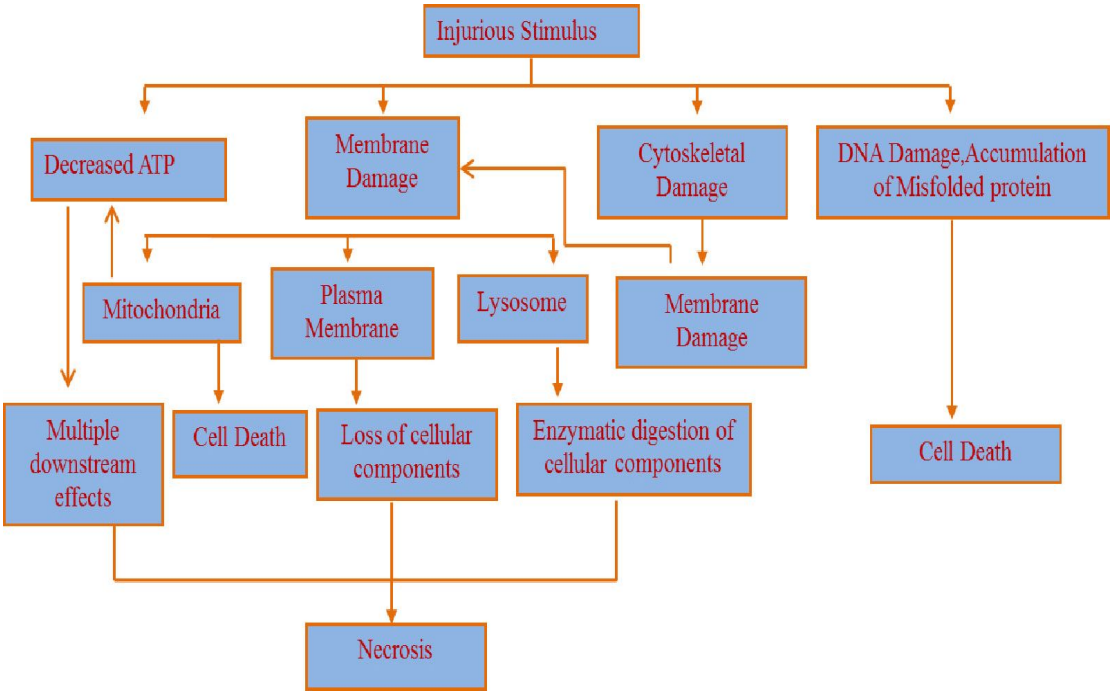
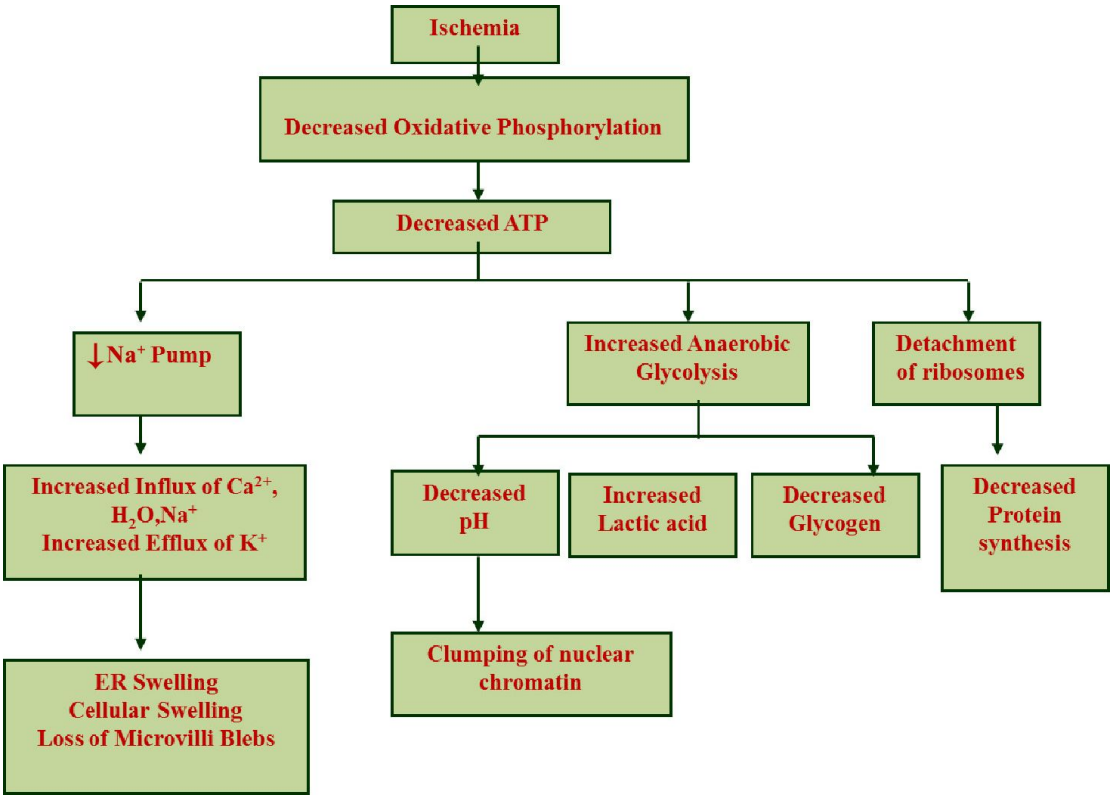


Figure : 9. Mechanisms Involved in Hypoxia-Ischemia Induced Cell Injury[55]

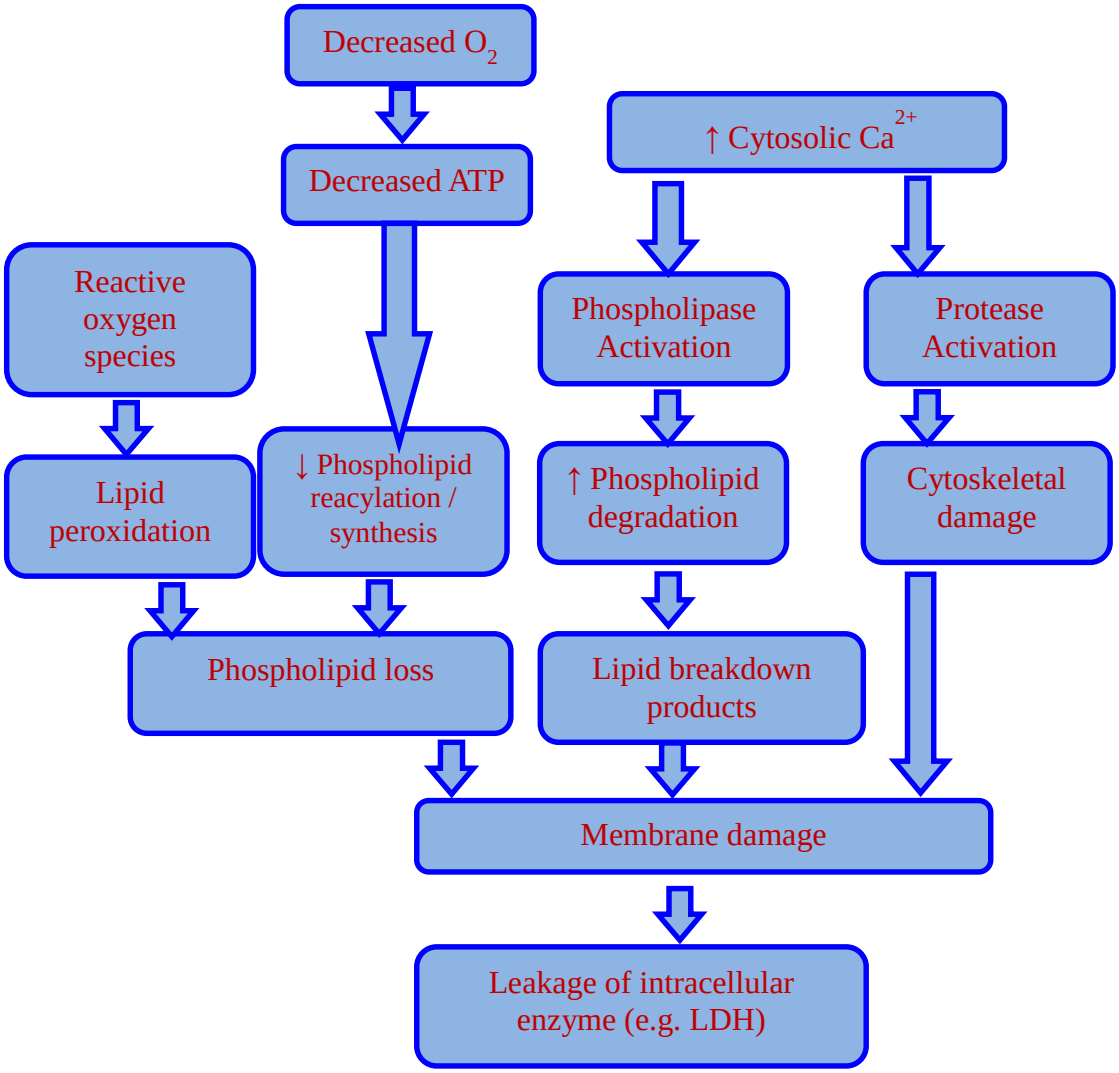


Hypoxia or ischemia will lead to decreased oxidative phosphorylation which leads to decreased ATP production. Decreased ATP production will lead to membrane injury.

Membrane Injury

The mechanism of cellular membrane damage is multifactorial. Failure of ATP dependent membrane bound Na+/k+ ATPase pump leads to depolarization of cells, allowing influx of Na+ and K+ ions with water causing cytotoxic neuronal edema. Calcium activates phospholipases and proteases with generation of oxygen free radicles lead to a breakdown in membrane phospholipids and cytoskeleton. Membrane phospholipid and cytoskeletons are an integral part of membranes. As the membrane lose their integrity, the damage become irreversible due to massive calcium influx and profound leakage of intracellular enzymes into the peripheral circulation. Calcium also contributes to the formation of oxygen free radicals by production of xanthine oxidase, nitric oxide and prostaglandins[55].

Figure : 10. Mechanisms of Membrane Damage in Cell Injury[55]



During severe asphyxia energy failure occurs leading to depletion of intracellular high energy phosphate compounds such as phosphocreatine and adenosine triphosphate. During anerobic conditions one molecule of glucose yields 38 molecules of ATP.

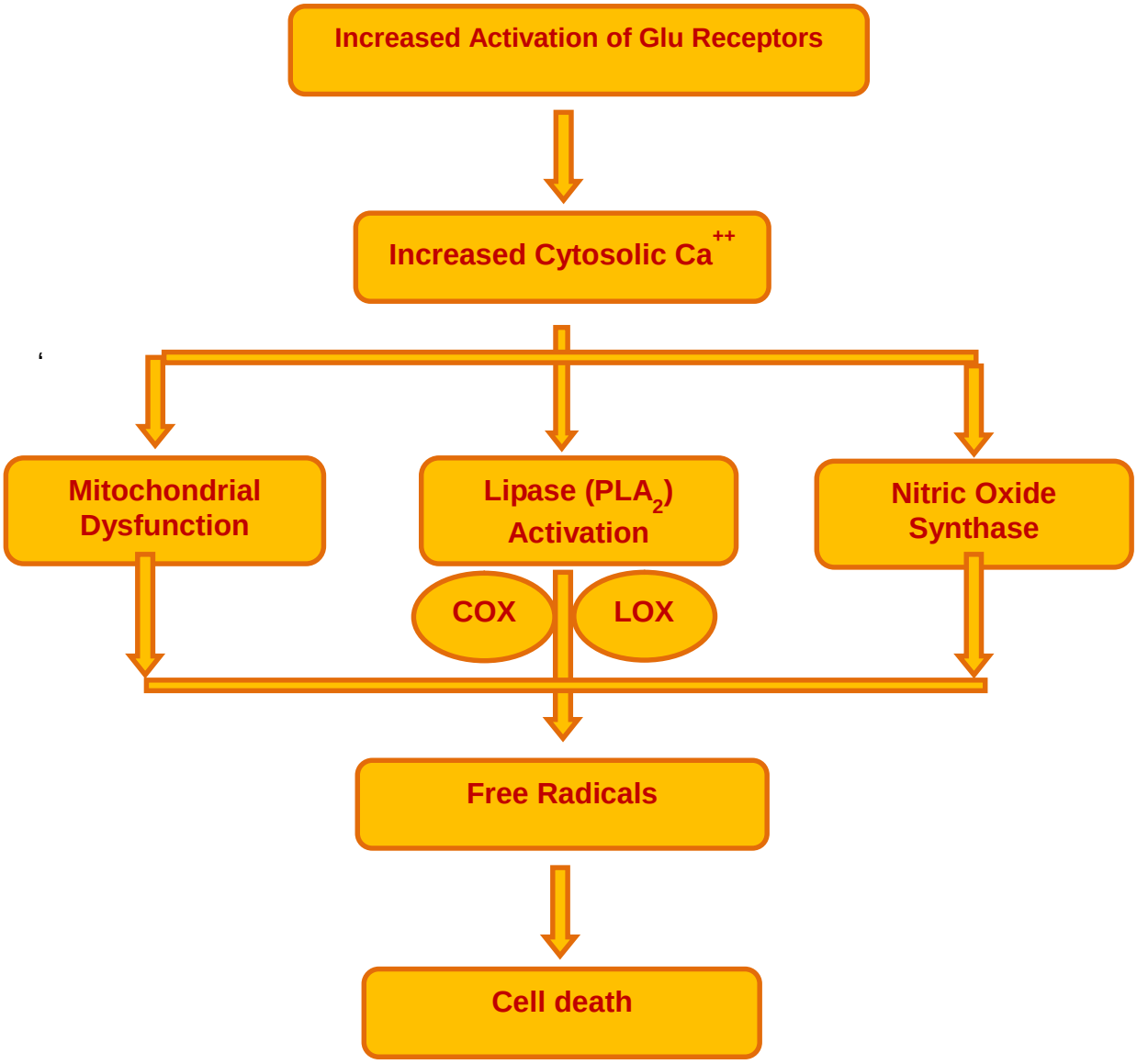
Reperfusion injury

During reperfusion, highly reactive oxygen derived free radicles are generated in many organs including brain. Free radicles are produced by oxygenation of arachidonic acid and hypoxanthine, and accumulation of nitric oxide. Naturally occurring oxygen free radicle scavengers try to limit the production of toxic radicles but these may be overwhelmed by the asphyxia. Due to secondary energy failure, there is ongoing neuronal injury in the area of brain adjacent to infarction. This peri-infarction area is called as penumbra[56].

Role of glutamate

Glutamate is one of the endogenous excitatory neurotransmitter in the brain. Asphyxia causes excessive release of glutamate from the presynaptic vesicles. The glutamate receptor is stimulated by NMDA which opens a receptor operated channel which allows calcium to enter the neurons causing further neuronal damage[56].

Figure: 11. Pathophysiology of Birth Asphyxia[57]



Specific Aspects of Pathophysiology of Birth Asphyxia

Hypoxia – ischemia causes a number of physiological and biochemical alterations[58]:

1. With brief birth asphyxia an alteration of heart rate occurs. There is transiently increase, followed by a decrease in heart rate (HR).With brief asphyxia there is mild elevation in blood pressure and an increase in central venous pressure (CVP) occurs. There is no change in cardiac output (CO).This alteration accompanied by a redistribution of cardiac output with increasing proportion of cardiac output going to the brain, heart and adrenal glands at the expense of reduction of perfusion to kidneys, lungs, gastro-intestinal tract, liver, spleen and skeletal muscles. This redistribution of cardiac output called Diving Sea Reflex. When there is severe but brief asphyxia as occurs in placental abruption, this diversion of blood flow to vital deep nuclear structures of the brain does not occur, results in the typical pattern of injury occurs to the subcortical and brain stem nuclei.

2. With prolonged birth asphyxia, there can be a loss of pressure auto regulation and or CO_2 vaso reactivity. This in turn leads to cerebral perfusion disturbances, particularly when there is cardiovascular involvement with hypotension or decreased cardiac output. A decrease in blood flow to the brain results in anaerobic metabolism and leads to

cellular energy failure due to increased glucose utilization in the brain and followed by fall in the concentration of glycogen, phosphocreatine and adenosine triphosphate. Prolonged duration of birth asphyxia results in diffuse cellular injury to both cortical and subcortical structures.

Organ Involvement in Birth Asphyxia

Central nervous system

The clinical spectrum of HIE described as mild, moderate, or severe (Sarnat and Sarnat stages of HIE[59]). EEG is useful to provide objective data to grade the severity of encephalopathy.

A. **Encephalopathy**. Newborns with HIE must have depressed consciousness by definition, whether mild, moderate, or severe. Mild encephalopathy consist of an apparent hyper alert or jittery state, but the neonate does not respond appropriately to stimuli, and consciousness is abnormal. Moderate and severe encephalopathies are characterized by more impaired response to stimuli such as light, touch or even noxious stimuli. The background pattern detected by EEG or aEEG is useful for determining the severity of encephalopathy[59].

B. **Brain stem and cranial nerve abnormalities**; Newborns with HIE may have brain stem dysfunction ,which manifest as abnormal or absent brain stem reflexes, including pupillary, corneal, oculocephalic, cough and gag reflexes. There can be abnormal eye movements such as

dysconjugate gaze, gaze preference, ocular bobbing or other abnormal patterns of bilateral eye movements and an absence of visual fixation or blink to light. Newborns may show facial weakness which are usually symmetric and have a weak or absent suck and swallow with poor feeding. They can have apnea or abnormal respiratory patterns.

c. **Motor abnormalities**. With severe encephalopathy, there is greater hypotonia, weakness and abnormal posture with lack of flexor tone, which is usually symmetric. With severe HIE, primitive reflexes such as moro or grasp reflex may be diminished. Over days to weeks the initial hypotonia may evolve into spasticity and hyperreflexia, if there is significant HI (Hypoxic-ischemic) brain injury. If a newborn shows significant hypotonia within the first day or so after birth, the HI insult may have occurred earlier in the antepartum and have established HI brain injury.

D. Seizures occur in up to 50% newborns with HIE and usually start within 24 hours after the HI insult. Seizures indicate that the severity of encephalopathy is moderate or severe, not mild.

Seizures may be subtle, tonic or clonic. It is sometimes difficult to differentiate seizures from jitteriness or clonus, although the latter two are usually suppressible with firm hold of the affected limb(s).

Since seizures are often subclinical (electrographic only) and abnormal movements or posture may not be seizure, EEG remains the gold standard for diagnosing neonatal seizures, particularly in HIE.

Seizures may compromise ventilation and oxygenation, especially in newborns who are not receiving mechanical ventilation.

Increased intracranial pressure resulting from diffuse cerebral edema in HIE often reflects extensive cerebral necrosis rather than swelling of intact cells and indicate a poor prognosis. Treatment to reduce ICP does not affect outcome.

To estimate the severity of asphyxial injury to newborns more than 36 weeks of gestational age Sarnat and sarnat clinical staging is used.

Table: 4. Sarnat and Sarnat Clinical Stages of Hypoxic –Ischemic Encephalopathy[59]

Stage	Stage1	Stage2	Stage3
Level of consciousness	Hyper alert; irritable	Lethargic or obtunded	Stuporous, comatose
Neuromuscular control	Uninhibited, Over reactive	Diminished spontaneous movement	Diminished or absent spontaneous movement
Muscle tone	Normal	Mild hypotonia	Flaccid
Posture	Mild distal flexion	Strong distal flexion	Intermittent decerebration
Stretch reflexes	overactive	Overactive, Disinhibited	Decreased or absent
Segmental myoclonus	Present or absent	Present	Absent
Complex reflexes	Normal	Suppressed	Absent
Suck	Weak	Weak or absent	Absent
Moro	Strong, low threshold	Weak, incomplete high threshold	Absent
Oculovestibular	Normal	Overactive	Weak or absent
Tonic neck	Slight	Strong	Absent
Autonomic function	Generalized sympathetic	Generalized parasympathetic	Both systems depressed
Pupils	Mydriasis	Miosis	Mid position, often unequal; poor light reflex

Respiration	Spontaneous	Spontaneous; occasional apnea	Periodic; apnea
Heart rate	Tachycardia	Bradycardia	Variable
Bronchial and salivary secretions	sparse	Profuse	Variable
Gastrointestinal motility	Normal or decreased	Increased diarrhea	Variable
Seizures	None	Common focal or multifocal (6 to 24 hours of age)	Uncommon (excluding decerebration)
Electroencephalographic findings	Normal (awake)	Early; generalized low voltage, slowing (continuous delta and theta)	Early periodic pattern with isopotential phases
		Later; periodic pattern (awake); seizures focal or multifocal;1.0 to 1.5 Hz spike and wave	Later; totally isopotential
Duration of symptoms	<24 hours	2 to 14 days	Hours to weeks
Outcome	About 100% normal	80% normal; abnormal if symptoms more than5 to 7 days	About 50% die; remainder with severe sequelae

Table: 5. Levene Grading of Hypoxic – Ischemic Encephalopathy[60]

Features	Mild	Moderate	Severe
Consciousness	Irritable	Lethargic	Comatose
Tone	some hypotonia	Moderate hypotonia	Severe hypotonia
Seizures	Nil	Present	Prolonged
Sucking/ respiration	Poor suck	Unable to suck	Unable to sustain spontaneous respiration

Mild encephalopathy

This stage is characterized by hyperalertness, staring, normal or decreased spontaneous activity and a lower threshold for all stimuli, including Moro reflux. Seizures are not a feature[59].

Moderate encephalopathy

Seizures occur commonly. This stage is characterized by lethargy, hypotonia, reduced spontaneous activity, a higher threshold for reflexes and mainly parasympathetic response. A differential tone is seen between the upper and lower limb, with the arms being relatively hypotonic compared to the legs[59].

Severe encephalopathy

These newborns are comatose, with hypotonia, and no spontaneous movements. Primitive reflexes and suck reflexes are absent. Seizures are frequent and prolonged; although in most severe cases there may be no seizure activity and isoelectric EEG[59].

Asphyxia is not the only cause of encephalopathy and alternative causes like hypoglycemia and meningitis must be considered and excluded before HIE used as a feature of postasphyxial insult.

Table: 6. Differential Diagnosis of Hypoxic Ischemic Encephalopathy[30]

Infective	Meningitis (viral or bacterial) Encephalitis
Trauma	Subdural hemorrhage
Vascular	Neonatal stroke
Metabolic	Hypoglycemia Hyper/ hyponatremia Bilirubin encephalopathy
Congenital malformation	Neuronal migration disorders
Neuromuscular disorder	Spinal muscular atrophy
Inborn error of metabolism	Urea cycle defects Pyridoxine dependency Lactate acidemias Amino academia (non – ketotic hyperglycemia) Organic academia
Maternal drug exposure	Acute or chronic

Gross changes in the brain involved in hypoxic ischemic encephalopathy[61]are:

1. Selective neuronal necrosis is the most common type of injury. This injury occurs more commonly in the neurons of the glial tissue. The regions at increased risk for injury are hippocampus, brainstem nuclei and basal ganglia, cerebellum.
2. Necrosis of thalamic nuclei and basal ganglia is a type of selective neuronal necrosis.
3. Focal or multifocal cortical necrosis: associated with cerebral edema with cystic encephalomalacia.
4. Watershed infarcts: occurs in boundary zones between two or more cerebral arteries. Watershed infarcts due to preferential blood flow to brain stem rather than cerebrum, leads to parasagittal cerebral injury in term and post term infants.

BRAIN IMAGING

Cranial ultrasound

Cranial ultrasound examination can demonstrate edema as loss of grey white differentiation when severe, but is generally insensitive for the detection of HI brain injury in the first days after birth. It may be useful to rule out large intracranial hemorrhage[62].

Computed tomography

When performed early after HI injury, CT is used to detect cerebral edema, hemorrhage and HI brain injury. CT may not be useful in predicting the sequelae in premature infants because there is excess water and low myelin in the premature brain which obscures gray white differentiation. CT is more useful in detecting cerebral edema in term infants[63].

Electroencephalopathy

Elecroencephalography is used to detect and monitor seizure activity and also define abnormal background patterns such as discontinuous burst suppression, low voltage or isoeletric patterns. EEG and evoked potentials along with the clinical signs useful to guide in evaluating and classifying the severity of the damage[64].

Magnetic Resonance Imaging (MRI)

Conventional T1 and T2 weighted MRI sequences are the best modality for determining the severity and extend of HI brain injury, but injury is not apparent on these sequences in the first days after the HI injury[64].

Diffusion –Weighted Imaging

Can show abnormalities within hours of an insult that may be useful in the diagnosis of neonatal HIE and an early indicator of possible brain injury. Normal findings on diffusion weighted imaging MRI between 2 and 18 days of age are associated with normal neuromotor outcome at 12 to 18 months. Abnormalities of deep grey matter that detected early associated with worse motor and cognitive outcomes. Abnormal DWI of the basal ganglia noted within 10 days of HI insult was associated with a 93% risk of abnormal neurodevelopmental outcome at 9 months to 5 years. The fetus copes with an asphyxia event by a number of productive reflexes to preserve the function of vital organs. Less well perfused tissues may be vulnerable to hypoxic ischemic injury[63].

Proton Magnetic Resonance Spectroscopy (MRS)

MRS measures relative concentration of various metabolites in tissue. Elevated lactate, decreased N-acetylaspartate and alterations of the ratio of these two metabolites in relation to choline or creatine can indicate HIE and help prognosticate neurological outcome[64].

MULTI OEGAN DAMAGE

Table :7. Multiorgan Systemic Effects of Birth Asphyxia[65,66]

System	Effect
Central nervous system	Hypoxic ischemic encephalopathy, infarction, intracranial haemorrhage, hypotonia, hypertonia, seizures, cerebral edema, long term neuromotor disability, apneic attacks
Cardiovascular system	Myocardial ischemia, decreased contractility, tricuspid regurgitation, pulmonary hypertension, Dysrhythmias
Lungs	Meconium aspiration, hyaline membrane disease, pneumonia, pneumothorax ,transient tachypnea, shock lung
Gastrointestinal	Bowel ischemia, necrotizing enterocolitis, GI bleeding, paralytic ileus and stasis, hepatic dysfunction
Renal	Acute tubular necrosis, Oliguria ,renal vein thrombosis
Adrenal	Adrenal hemorrhage
Hepatic	Elevation of hepatocellular enzymes, hypoglycemia, altered metabolism or elimination of drugs
Hematology	Disseminated Intravascular coagulation, thrombocytopenia, hyperbilirubinemia
Metabolic	Hypoglycemia, hyponatremia, hypocalcemia, acidosis, Hyperkalemia
Immunologic	Septicemia
Endocrine	Syndrome of inappropriate secretion of antidiuretic hormone, transient hypoparathyroidism

Lungs

During intrapartum asphyxia, the fetus passes meconium and gasping occurs due to brainstem compromise. The gasp causes meconium to be aspirated in to the bronchial tree and may cause a chemical pneumonitis with severe pulmonary hypertension and a neonate at high risk of air leak. Infants mechanically ventilated for meconium aspiration syndrome will not show clinical signs of encephalopathy and cerebral injury may not be recognized. These newborns should have continuous EEG monitoring to assess cerebral function[67].

Cardiovascular system

Cardiac dysfunction caused by transient myocardial ischemia. Blood flow to the myocardium is preserved during asphyxia but cardiac compromise is a relatively common complication of hypoxic ischemic injury[3]. Myocardial dysfunction detected by Doppler ultrasound studies reported in 28-40% of asphyxiated newborns. Recognized complications include cardiogenic shock and hypotension, arrhythmias, functional tricuspid incompetence secondary to acute cardiac dilatation and myocardial ischemia which may be diagnosed from the electrocardiogram. The electrocardiography may show ST depression in the mid precardium and T wave inversion in the left precordium. Echocardiographic findings include decreased left ventricular

contractility, especially of posterior wall; elevated ventricular end diastolic pressure; tricuspid insufficiency and pulmonary hypertension. In severely asphyxiated newborns, dysfunction more commonly affects the right ventricle. A fixed HR may raise suspicion of severe brain stem injury[4].

Renal impairment

The kidney is the organ most commonly affected in asphyxial injury[4]. The proximal tubule of the kidney is commonly affected due to decreased perfusion. Oliguria and acute tubular necrosis occurs following birth asphyxia. This usually recovers with supportive treatment alone. The incidence of oliguria after perinatal asphyxia occurs in 23%-55% of babies and acute kidney injury was reported in 19% of asphyxiated newborns[68]. Acute retention of urine is a common complication following perinatal asphyxia and indicates severe cerebral injury. Renal failure following asphyxia also reported due to myoglobinuria.

Gastrointestinal tract

Gastrointestinal effects include an increased risk of bowel ischemia and necrotizing enterocolitis[67].

Metabolic disorders

One of the common complications of birth asphyxia is inappropriate antidiuretic hormone secretion with concentrated urine and hyponatremia[67].

Hematological disorders

Disseminated intravascular coagulation (DIC) is the hematological complication of birth asphyxia[67]. The clinical features of DIC are excessive bleeding from puncture sites and petechial hemorrhages. The pathophysiology of DIC is due to damage to blood vessels and poor production of platelets by the bone marrow. Due to liver dysfunction and poor synthesis of clotting factors leads to DIC.

A study conducted by **Arkhngel'skii et al** in 1996 reported DIC in 120 autopsy cases on neonate who died of intraventricular hemorrhage, asphyxia, respiratory distress and congenital pneumonia[69].

The combined criteria for MOD are based on clinical and biochemical measurements and differ between different studies[3,65,66].

Criteria for MOD in infants with Birth asphyxia used by **shah et al**[65].

1. Renal

i) Presence of anuria or oliguria (<1ml/kg/hr) for 24 hours or more

ii) Serum creatinine concentration > 100 mmol/L

iii) Presence of anuria/oliguria for >36 hrs

iv) Any serum creatinine level >125 mmol /L

v) Serial increase in serum creatinine values postnatally.

Cardiovascular

i) Hypotension requiring inotrope support to maintain blood pressure within the normal range for more than 24 hour.

ii) Electrocardiographic evidence of transient myocardial ischemia.

Pulmonary

If patient needs ventilator support with 40% of oxygen requirement for first four hours after birth.

Hepatic

Elevation of hepatic enzymes such as AST, ALT >100 U/L at any time during the first week after birth.

The investigative indicator

Electrocardiography

Earliest findings linking fetal hypoxia with ischemic changes on prenatal electrocardiogram came from southern (1957)[70]. He suggested that low oxygen saturation at birth was associated with prolonged PR interval and prolonged QRS and ST segment duration. T wave inversion is seen in 35% of the anoxic cases[71].

In 14 patients who had tricuspid insufficiency, Buciareli[72] in 1977 reported ST depression in anterior precordial leads. These changes may last for months after resolution of murmur.

A conclusive diagnosis of myocardial necrosis can be made only by histological examination of newborn that die after 24 hours of age. Histological findings of the heart reported after asphyxia include myocardial and subendocardial hemorrhage, necrosis, cardiomyopathy, and infarction of the endocardial muscle. Most babies with ischemia may survive. Hence to determine true and false negative ECG is deficient. The ischemic damage due to birth asphyxia may be identifiable on ECG but clinical outcome not directly related to ECG changes.

In summary the ECG changes following perinatal asphyxia are transient. More specific is the ST and Q wave changes may not correlate with severity of dysfunction[71].

CK-MB

Creatine phosphokinase is an enzyme expressed by various cell types and tissues. Creatine kinase is an enzyme which catalyzes the conversion of creatine to phosphocreatine. It also converts ATP into ADP. This reaction is reversible. ATP can be generated from phosphocreatine and ADP[73].

In cells and tissues that consume ATP rapidly, especially brain, photoreceptor of the retina, spermatozoa, skeletal muscle, hair cells of the inner ear and smooth muscle. In the body phosphocreatine acts as an energy reservoir for rapid buffering and regeneration of ATP as well as for Intracellular energy transport by the phosphocreatine shuttle or circuit.

Types of CK-MB

Based on their location CK-MB may be cytosolic or mitochondrial. Cytosolic CK-MB enzyme consists of two subunits which are B (brain type) or M (muscle type). There are three types of isoenzymes; CK-MM, CK-BB and CK-MB. The genes for subunits located on different chromosomes. M located on 19q13 and B located on 14q32.The ubiquitous and sarcomeric form are two types of mitochondrial isoenzymes[74].

Table: 8. Shows the Normal Values of CK and its Isoenzymes[75]

Age	CK	MB%	BB%
Cord blood	70-380U/L	0.3-3.1	0.3-10.5
6-8 hour	214-1175 U/L	1.7-7.9	3.6-13.4
24-33 hour	130-1200 U/L	1.8-5.0	2.3-8.6
72-100 hour	87-725 U/L	1.4-5.4	5.1-13.3
Adult	5-130 U/L	0-2	0

The upper limit of the normal range of CK-MB at 6-8 hours of life is 7.9% of 1,175 U/L which is 92.6 U/L.A serum CK-MB value >92.6U/L at 8 hours is abnormal[75].

The sites of storage of CK in the body are the heart muscle (CK-MB), brain (CK-BB) and skeletal muscle (CK-MM). The isoenzyme pattern differs in tissues. The heart muscle (myocardium), expresses CK-MM at 70% and CK-MB at 25-30%. CK-BB is expressed in all tissues at low levels and has little clinical relevance. Skeletal muscle expresses CK-MM (98%) and low levels of CK-MB (1%). CK-BB occurs mainly in tissues and its levels rarely have any significance in bloodstream.

Creatinine kinase assayed as a marker of myocardial infarction. It also used as a marker of muscular dystrophy, rhabdomyolysis, acute renal failure and in autoimmune myositis. Transient myocardial ischemia may occur in any newborn with a history of birth asphyxia. An elevated serum CK-MB or cTnT may be helpful in determining the myocardial injury. In

myocardial injury elevation of CK-MP fraction 5% to 10% may be seen[4]. In birth asphyxia with acidosis there is leakage of CK and its isoenzymes from the damaged cells. Hence a marked elevation of the enzyme values may indicate a poor prognosis[76].

In 1985 a study was conducted by **primhak et al[17]** and showed CK-MB in normal and asphyxiated neonates peaked at 8 hours and fell by 72 hours. The study stated that Absolute and percentage level of CK-MB were higher in asphyxiated babies. They concluded that myocardial injury in newborn is associated with CK-MB release, but in view of lack of cardiac specificity of CK-MB caution is urged during the interpretation of elevated enzyme activity in newborn.

Fonseca E et al[20] in 1995 showed that antepartum fetal distress is associated with release of enzymes such as CK-BB and CK-MB. These biochemical markers may indicate either brain or myocardial damage. Total CK and its isoenzyme activity was measured in cord blood and 24 hours after birth in peripheral blood. Abnormal FHR patterns associated with elevated enzyme activities. Total CK and its isoenzymes levels are higher in asphyxiated newborns when compared to non - asphyxiated neonates. Electrocardiography showed ischemic changes in seven neonates had higher CK-MB and CK-BB level both at birth and within 24

hours after birth. In the neonate with abnormal fetal heart rate, CK-MB and CK-BB were increased with predominance of CK-MB.

In 1999 **Barberi et al**[22] reported that CK, CK-MB, CK-MB/CK ratio and LDH were all increased in an asphyxiated group. The study reported that in a babies with respiratory distress only CK-MB, CK-MB/CK ratio were abnormal.

Jedeiken et al[77] reported that peak levels of all isoenzymes of CK-MB seen at 5-33 hours post-natally.

Omokhodion SI et al[19] studied the CK and CK-MB levels in 23 asphyxiated neonates and 12 healthy controls during the first 100 hours of life. The asphyxiated neonates showed elevated mean CK and CK-MB levels. Peak mean CK and CK-MB values were 789.17±220 U/L and 16.36±3.0 U/L respectively with P value less than 0.001.

Boo NY et al[24], showed, asphyxiated neonates had higher concentrations of cTnT and CK-MB. Serum cTnT concentrations were significantly higher in asphyxiated neonates who died or developed cardiac dysfunction.

Lactate Dehydrogenase

LDH is an enzyme which exist in four enzyme classes. Two classes are cytochrome dependent enzymes. They are acting on either L-lactate or D-lactate. The other two classes are nicotinamide adenine dinucleotide dependent enzymes with each acting on D –Lactate or L-Lactate[27].

L-lactate dehydrogenase is a tetrameric enzyme has four subunits occur in two isoforms, designated M (for muscle) and (H for heart). The subunits can combine as shown below to yield isoenzymes of LDH[78].

Lactate Dehydrogenase Isoenzymes	Subunits
I1	HHHH
I2	HHHM
I3	HHMM
I4	HMMM
I5	MMMM

Heart muscle expresses the H sub unit exclusively, isoenzyme I1 predominates in heart muscle. Isoenzyme I5 predominates in liver. Small quantities of LDH are normally present in plasma. LDH is abundant in red blood cells.LDH 2 predominantly seen in reticuloendothelial cells. Isoenzyme 4 predominates in kidney and pancreas. Following myocardial injury or liver dysfunction, the damaged tissues release LDH isoforms

into the blood[79]. The elevation of isoenzymes are detected by separating the different oligomers of LDH by electrophoresis and assaying their catalytic activity. Tissue breakdown elevates levels of LDH.

The normal reference value of LDH in infants < 1 year is 170-580U/L. A value > 580 U/l at 72 hours is abnormal[75].

Leakage of intracellular enzymes such as LDH signalling multiorgan damage is seen together with HIE after birth asphyxia. In hypoxic hepatitis, peak concentration of aminotransferases and LDH is seen within 24 to 72 hours after the insult.

Lackmann et al[80] study showed LDH and AST during first 72 hours post partum predict HIE or intravantricular hemorrhage in term and preterm infants. In their study 10 out of 49 term infants developed HIE and sensitivity and specificity for prediction of HIE were 90% and 71% respectively.

Sanchez Nava et al[18] study showed that AST, ALT and LDH were raised among asphyxiated newborns.

In 2010, **Karlsson M et al**[27] study was done in 2008 on evaluation of organ damage in asphyxia concluded that a cut off value of 1049 U/L

for LDH was the most suitable predictor of mild ,moderate and severe HIE with sensitivity of 100% and specificity of 97%.

Reddy et al[25] study showed that LDH had 100% specificity for asphyxia. The mean LDH value at 72 hours was 1109.5± 520.6 in cases and 231.5± 177.5 U/L in controls.

Reason for Planning the Study

Birth asphyxia presents as multiorgan injury and the newborn could manifest with encephalopathy, seizures, respiratory distress, cyanosis, renal failure and feeding intolerance. The signs and symptoms overlaps with other illnesses like sepsis, heart disease, pneumonia, and heart failure etc. requiring different management with different prognosis. In the absence of observation at birth or history of failure to breath the diagnosis of birth asphyxia based on signs and symptoms difficult. So establishment of correct diagnosis is important for immediate management and long term prognosis.

MATERIALS AND METHODS

Source of Data

The study was a prospective study conducted on asphyxiated and non asphyxiated neonates recruited from neonatal intensive care unit and post natal ward of Govt. Mohan Kumaramagalam Medical College & hospital from June 2013 to may 2014. The blood samples from the 50 neonates comprising the cases and 50 neonates comprising the controls constituted the material for the study.

Method of Collection of Data

The study included two groups:

The case group: It included 50 neonates fulfilling the following criteria:

Inclusion criteria:

1. Gestational age ≥37 weeks
2. Appropriate for gestational age
3. The neonates will be identified to have experienced perinatal asphyxia when at least 3 of the following are present.
    a) Intrapartum signs of fetal distress, as indicated by non reassuring NST on continuous electronic fetal monitoring and/ or by thick meconium staining of the amniotic fluid.
    b) Apgar score of <7 at one minute of life.

c) Resuscitation with >1 minute of positive pressure ventilation before stable spontaneous respiration

d) Mild, moderate or severe hypoxic ischemic encephalopathy as defined by sarnat & sarnat[59] staging

Exclusion criteria

1. Congenital malformation
2. Maternal drug addiction
3. Neonates born to mother who had received magnesium sulphate within 4 hours before delivery or opiods (pharmacological depression)
4. Hemolytic disease of the newborn

The control group

It included 50 term apparently healthy neonates appropriate for gestational age without signs of perinatal asphyxia as evidenced by normal fetal heart patterns, clear liquor and one minute apgar score≥ 7

All newborns included in the study had the following done

1. Informed consent obtained from the mother
2. Detailed maternal history, intrauterine fetal well being assessment by continuous electronic fetal monitoring, birth events, meconium staining amniotic fluid, apgar score, sex and weight of the baby are

recorded on the proforma. Gestational age was assessed by new ballard scoring system.

3. Thorough clinical and neurological examination was done for all the neonates included in the study. The asphyxiated newborns (case group) were monitored for seizures, HIE in the immediate neonatal period in the NICU. A clinical grading system by Sarnat and Sarnat[59] was used to grade the severity of HIE.
4. Blood sample were collected from the newborn and sent for:
    a) Creatine kinase muscle brain fraction (CK-MB) levels.
    b) Lactate dehydrogenase (LDH)levels

Blood for Creatine Kinase –Muscle Brain fraction was drawn at 8±2 hours. Blood for Lactate dehydrogenase was drawn at 72±2 hours of age. Laboratory technicians performing the CK-MB and LDH tests were masked to the identity and birth history of asphyxia of the newborn.

The upper limit of the normal range of CK-MB at 8 hours of life is 7.9% of 1,175U/L which is 92.6U/L. A serum level of CK-MB >92.6 U/L at 8 hours of life was taken as the cut off level.

The normal reference value of LDH in newborns and infants <1 year is 170-580 U/L.A value >580 U/L at 72 hours taken as the cut off level.

5. The case group had other investigations and imaging studies as required for post resuscitation management of asphyxiated newborns. The causes for hypotonia, seizures, lethargy, poor feeding, other than HIE were ruled out with relevant investigation available. Peripheral smear study for RBC morphology and reticulocyte count was used to document hemolytic disease of the newborn.

Sample Collection

Serum CK-MB

1 ml of blood was collected each time from peripheral venous site at 8 hours of age. Blood sample was collected in sterile bottle, sent immediately to the biochemistry laboratory of the hospital. Blood was checked for any hemolysis. No anticoagulant was added as they inhibit CK-MB activity.

Method of Estimation of CK-MB

CK-MB analysis was done by immuno-inhibition method using the kit manufactured by Awareness technology LTD in technical collaboration with CLINIQA CORPORATION United states of America. The device used was CHEMWELL 902.

Test Principle and Procedure

The test utilizes immune inhibition method and is a quantitative method. The reagent contains a polyclonal antibody to the monomer of the CK-MB .The antibody completely inhibits the activity of CK-MM and half of the activity of CK-MB. The activity of non-inhibited B monomer of CK-MB is measured as a serum value which represents half the activity of CK-MB. The activity of CK-BB isoenzyme is zero.

In this method serum is added to a modified CK-NAC reagent that contains the anti M antibody.

The activity of CK-MB is determined using the following reaction sequence;

$$\text{Creatine phosphate} + \text{ADP} \xrightarrow{\text{CK}} \text{Creatine} + \text{ATP}$$

$$\text{ATP} + \text{Glucose} \xrightarrow{\text{HK}} \text{G-6-P} + \text{ADP}$$

$$\text{G-6-P} + \text{NADP}^{+} \xrightarrow{\text{G-6-P DH}} \text{6-PG} + \text{NADPH} + \text{H}$$

The rate of increase in absorbance at 340 nm resulting from formation of reduced Nicotinamide adenine dinucleotide phosphate (NADPH) is followed with time and is a measure of the enzymatic activity.

Serum LDH

1 ml clotted blood was taken in the peripheral line and was analysed in the main biochemistry lab by immunoassay tests. Hemolysed samples were not analysed because of increased LDH values were observed due to increased RBC destruction. The normal reference value of LDH in newborns and infants <1 year is 170-580 U/L.A value >580 U/L at 72 hours taken as the cut off level.

Method of Estimation of LDH

LDH analysis was done by immuno-inhibition method using the kit manufactured by Awareness technology LTD in technical collaboration with CLINIQA CORPORATION United states of America. The device used was CHEMWELL 902.

Test Principle and Procedure

The test utilises DGKG method which is a quantitative method. This method is a standard method according to the recommendations of the International federation of clinical chemistry.

LDH catalizes the following reaction:

$$\text{Pyruvate} + \text{NADH} + H^{+} \xrightarrow{\text{LDH}} \text{L-Lactate} + \text{NAD}$$

The absorbance value at 340 nm, due to the NADH oxidation to NAD, is directly proportional to the enzyme activity which is determined photometrically.

Figure: 12. Chemical Auto Analyzer with Standardized Kit Performing Enzyme assay

Statistical Methods

Descriptive statistical analysis has been carried out in the present study. Results are presented as Mean ± SD (Minimum-Maximum). Results on categorical measurements are present in number (%). Significance is assessed at 5% level of significance. Student t test has been used to find the significance of the study parameters between two study groups. Sensitivity, specificity and positive predictive value and negative predictive value of the test were performed.

RESULTS AND OBSERVATIONS

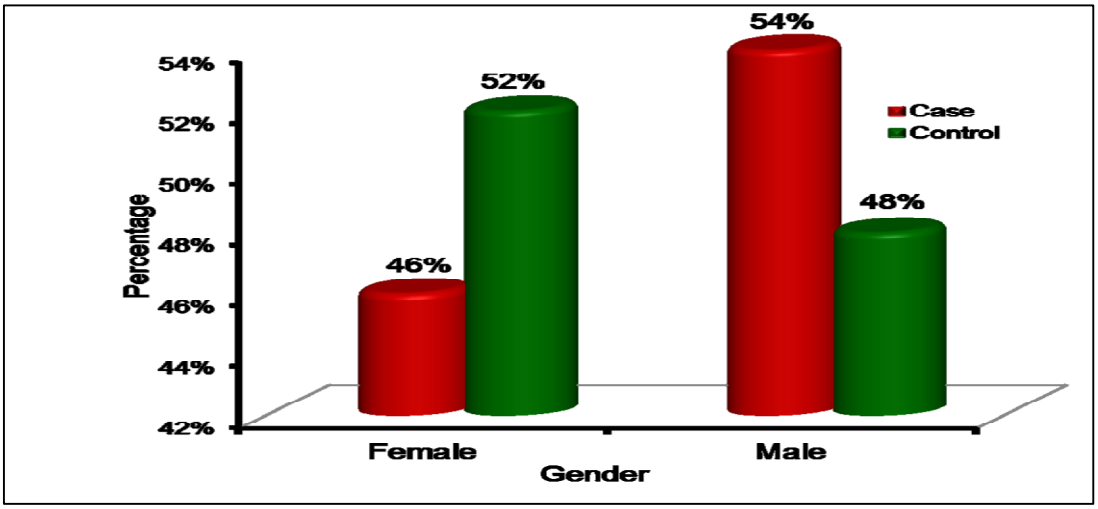
The study was a prospective study conducted on asphyxiated and non asphyxiated newborns recruited from neonatal intensive care unit and post natal ward in Government Mohan Kumaramangalam Medical College & Hospital from June 2013 to May 2014. Total number of study group was 100. Fifty cases were studied with documented birth asphyxia. Fifty controls were neonates without birth asphyxia.

Table : 9. Shows Gender Distribution of Neonates Studied

Gender	Cases (n=50)		Controls (n=50)	
	No	**%**	**No**	**%**
Female	23	46	26	52%
Male	27	54	24	48%
Total	**50**	**100**	**50**	**100**

P –Value 0.320 > 0.05 No difference

Figure: 13. Shows General Distribution of Neonates Studied



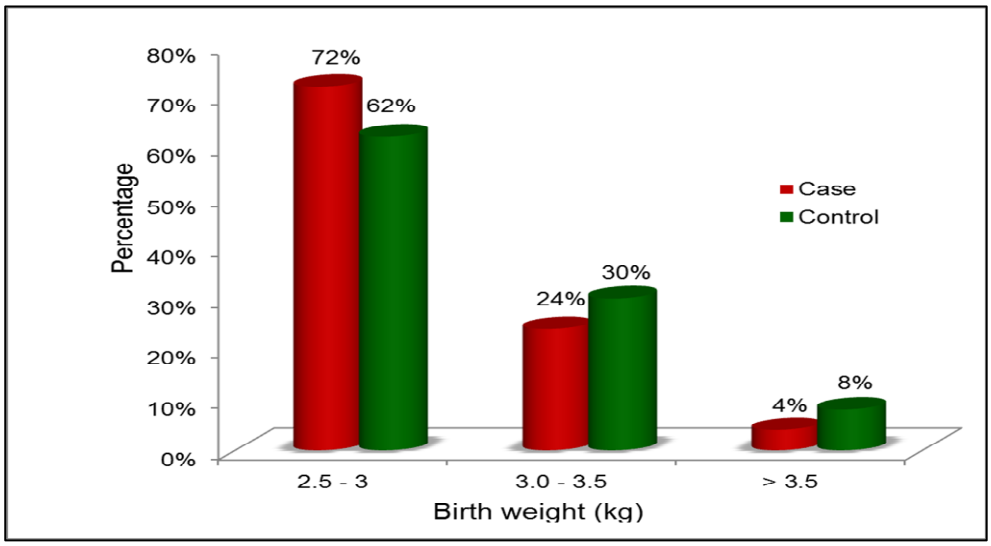
Among the 50 neonates in case group, there were 27 (54%) males and 23 (46 %) females. Among the 50 neonates in control group 26 (52%) males and 24 (48%) females. Gender distribution of neonates is statistically similar between two groups with p=0.320

Table: 10. Shows Birth Weight Distribution of Neonates Studied

Birth Weight(kg)	Cases (no=50)		Controls (no=50)	
	No	%	No	%
2.5 – 3.0	36	72	31	62
3.0 - 3.5	12	24	15	30
> 3.5	2	4	4	8
Total	**50**	**100**	**50**	**100**
Mean ± SD	2.8±0.4		2.9±0.5	

P –Value 0.552 > 0.05 No difference

Figure: 14. Shows Birth Weight Distribution of Neonates Studied



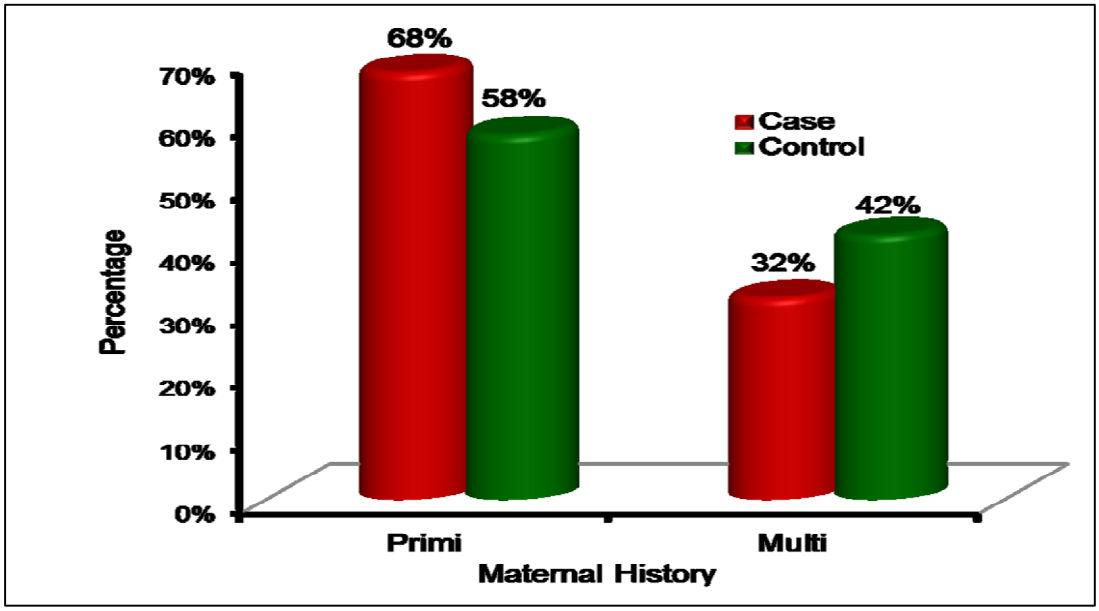
Among the neonates in case group 36 (72%) neonates weighed between 2.5-3.0 kg, 12(24%) weighed between 3.0 to 3.5 kg and 2 (4%) weighed >3.5 kg. Among the neonates in control group 31 (62%) neonates weighed between 2.5-3.0 kg, 15 (30%) weighed between 3.0 to 3.5 kg and 4(8%) weighed >3.5 kg. The mean weight in case group was2.8±0.4 kg and in control group was 2.9±0.5kg. Birth weight distribution is statistically similar with p=0.552

Table :11. Shows Maternal History of Neonates Studied

Maternal History	Cases (n = 50)		Controls (n = 50)	
	No	%	No	%
Primi	34	68	29	58
Multi	16	32	21	42
Total	**50**	**100**	**50**	**100**

P –Value 0.130 > 0.05 Significant

Figure: 15. Shows Maternal History of Neonates Studied

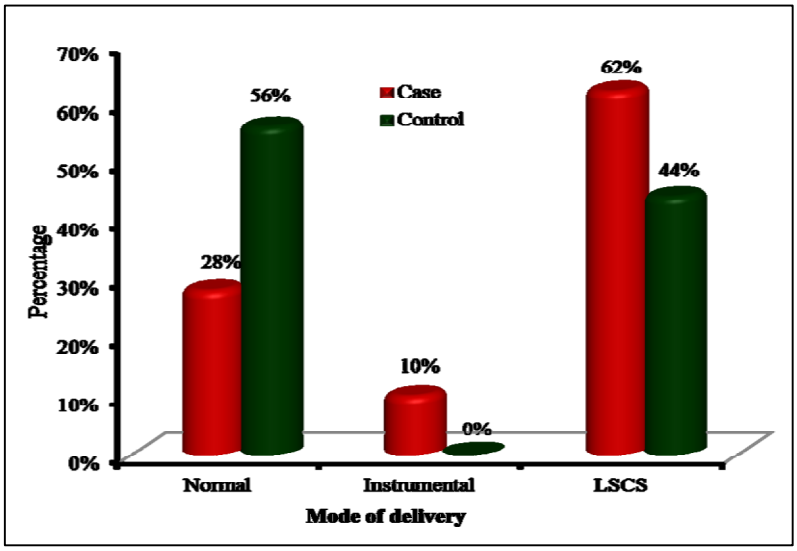


Among the 50 neonates in case group, 34 (68%) were born to primi mothers and 16 (32%) were born to multi gravida mothers. Among the control group of 50 neonates, 29 (58 %) were born to primi mothers and 21 (42%) to multi gravida mothers. Proportion of primi and multi gravida mothers are statistically similar with P = 0.130.

Table :12. Shows Mode of Delivery of Neonates Studied.

Mode of delivery	Cases(no=50)		Controls(no=50)	
	NO	**%**	**NO**	**%**
Normal	14	28	28	56
Instrumental	5	10	0	0
LSCS	31	62	22	44
Total	**50**	**100**	**50**	**100**
Inference	Incidence of caesarean section and instrumental delivery more in cases compared to controls with p value < 0.001			

Figure: 16. Shows Mode of Delivery of Neonates Studied

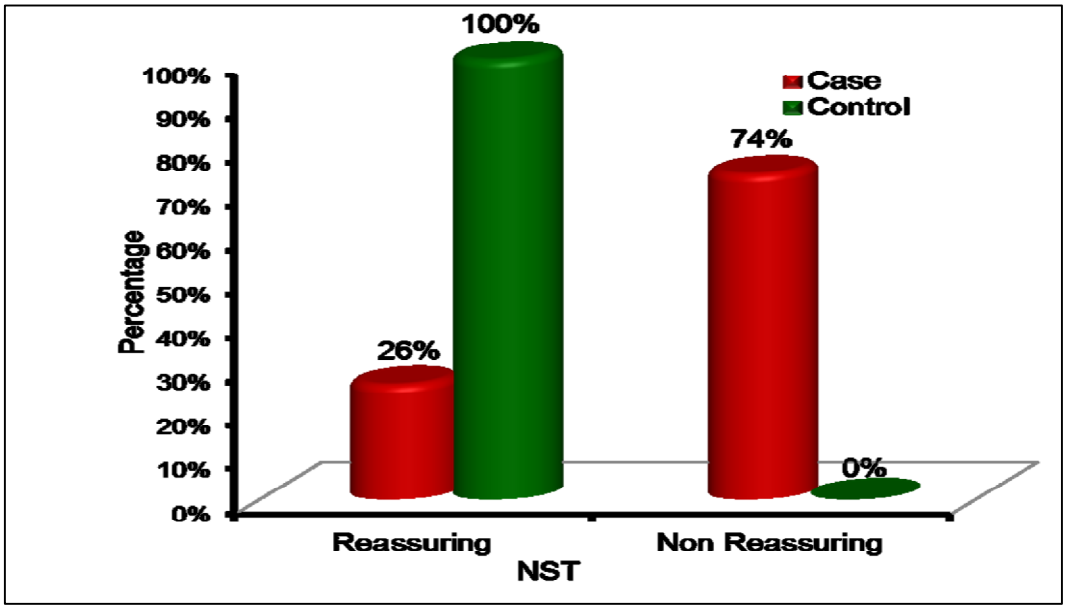


Among 50 neonates in case group, 14 (28%) neonates were delivered by normal delivery, 5 (10%) neonates were delivered by instrumental delivery and 31 (62%) delivered by cesarean delivery. Among 50 neonates in control group 28 neonates were delivered by normal delivery and 22 delivered by cesarean section. Incidence of caesarean section and instrumental delivery more in case group (72%) compared to control group (44%) with p value < 0.001

Table :13. Shows Non Stress Test (NST) of Neonates Studied

NST	Cases (No=50)		Controls (No=50)	
	No	**%**	**No**	**%**
Reassuring	13	26	50	100
Non Reassuring	37	74	0	0
Total	**50**	**100**	**50**	**100**
Inference	Incidence of non reassuring NST significantly more in case group against control group with p value <0.001			

Figure :17. Shows Non Stress Test (NST) in Neonates Studied

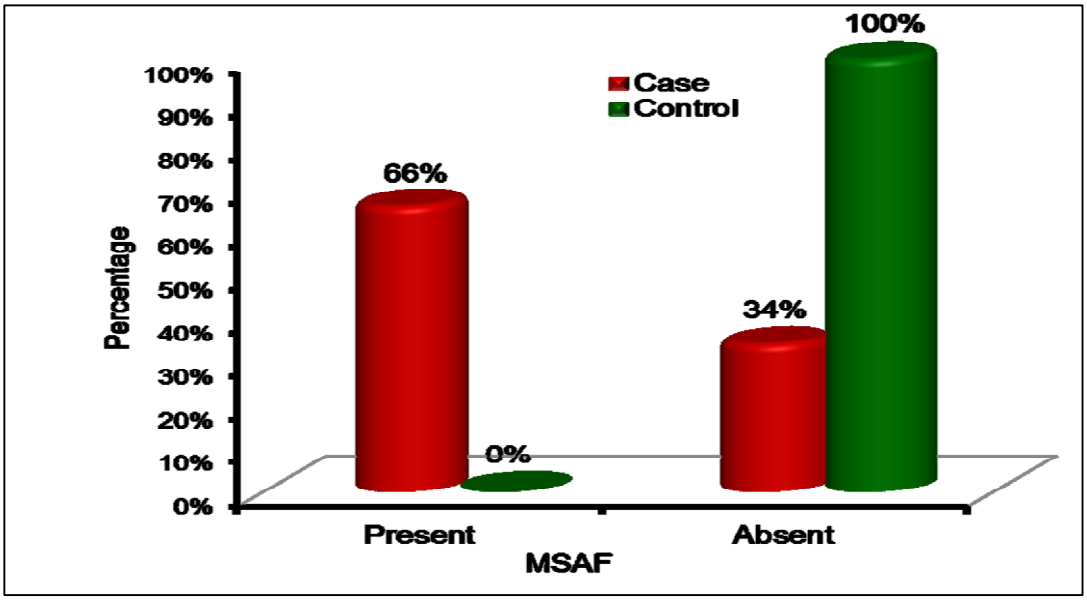


Among the 50 neonates in case group 13 cases (26%) had reassuring NST and 37 cases (74%) had non reassuring NST. Non reassuring NST indicates fetal distress. All neonates in control group had reassuring NST. Incidence of non reassuring NST significantly more in case group against control group with p value <0.001

Table :14. Shows Meconium Stained Amniotic Fluid (MSAF)in Neonates Studied

MSAF	Cases(n=50)		Control(n=50)	
	No	**%**	**No**	**%**
Present	33	66	0	0
Absent	17	34	50	100
Total	**50**	**100**	**50**	**100**
Inference	Incidence of MSAF is significantly more in cases when compared to controls with p value <0.001			

Figure :18. Shows Meconium Stained Amniotic Fluid in Neonates Studied

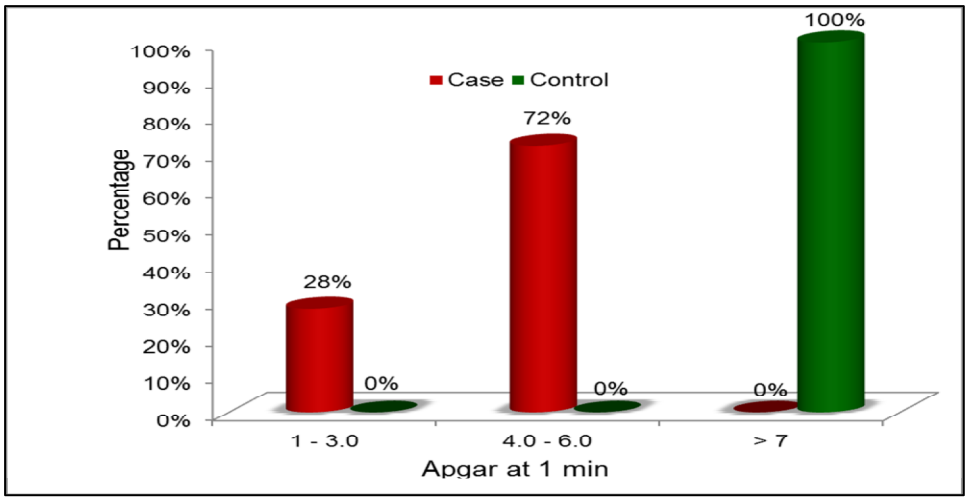


Among the 50 neonates in case group 33 (66%) had thick MSAF and 17 (34%) had clear liquor. All neonates in control group had clear liquor. Incidence of MSAF is significantly more in cases when compared to controls with p value < 0.001

Table :15. Shows Comparison of Apgar Score in Neonates Studied

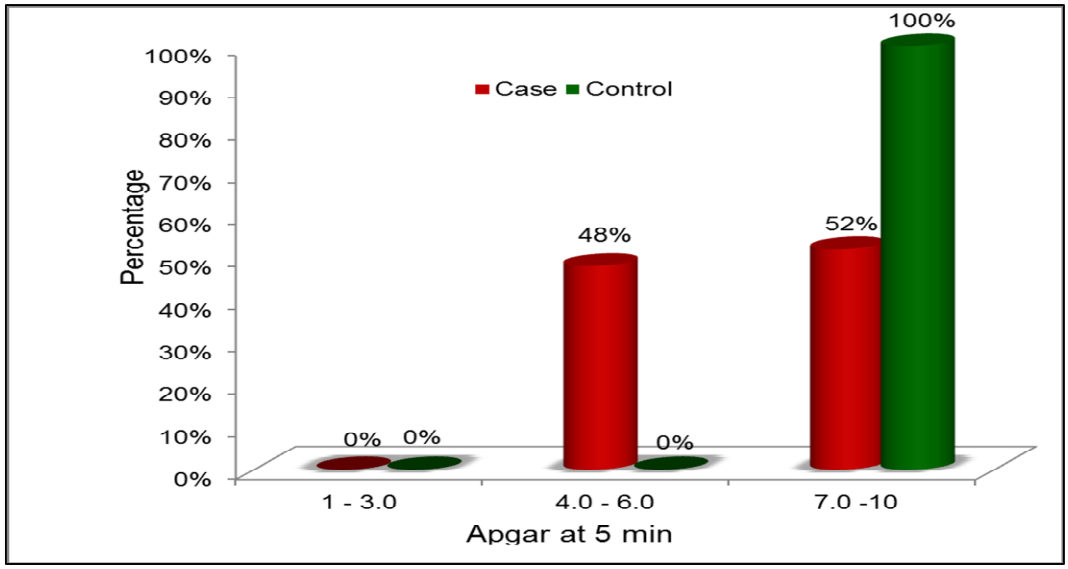
Apgar score	Cases(no=50)		Controls(no=50)		P Value
	No	%	No	%	
1 minute apgar score					
0-3	14	28	0	0	<0.001**
4-6	36	72	0	0	
≥ 7	0	0	50	100	
Total	**50**	**100**	**50**	**100**	
5 minute apgar score					
0-3	0	0	0	0	<0.001**
4-6	24	48	0	0	
≥7	26	52	50	100	
Total	**50**	**100**	**50**	**100**	

Figure :19. Shows Comparison of Apgar Score at 1 min in Neonates Studied



Among the 50 neonates in case group, all the 50(100%) neonates had an Apgar score of <7 at 1 min. 14 (28%) cases had an apgar score between 0-3(severe birth asphyxia) and 36 (72%) cases had apgar score between 4-6 (moderate birth asphyxia). All the neonates in control group had an apgar score ≥ 7. Incidence of apgar score <7 is significantly more in cases at 1 min with p<0.001

Figure :20. Shows Comparison of Apgar Score at 5 min in Neonates Studied

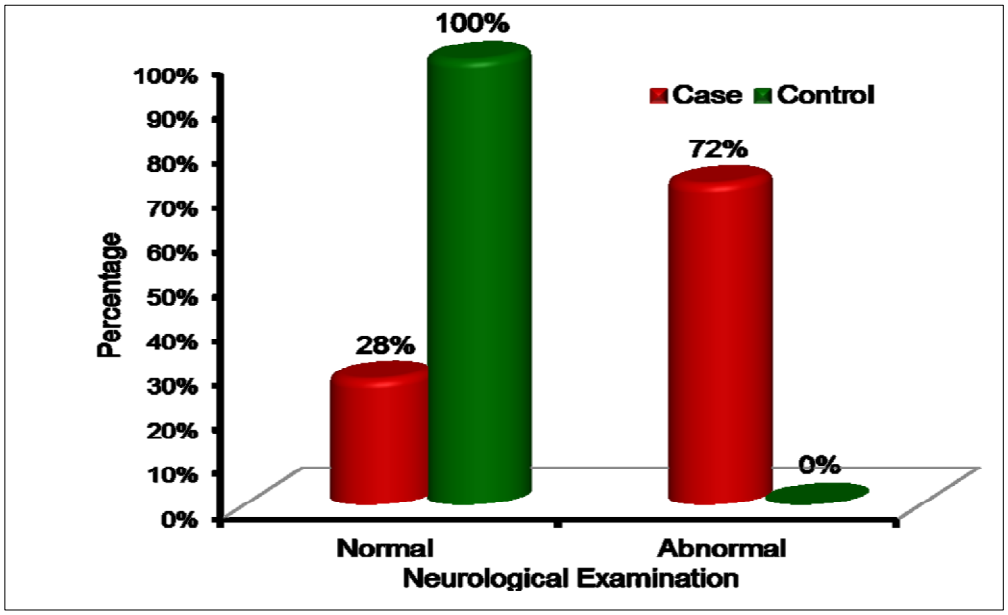


Among the 50 neonates in case group 26(52%) neonates had an apgar score of ≥ 7 at 5 min following resuscitation at birth.24(48%) had an apgar score <7 at 5 min even with resuscitation.All the 50 (100%) neonates in control group had an apgar score >7 at both 1 and 5 min. Incidence of apgar score <7 at 5 min is significantly more in cases at with $p<0.001$

Table :16. Shows Neurological Examination of Neonates Studied

Neurological examination	Cases		Control		P Value
	No	%	No	%	
Normal	14	28	50	100	< 0.001**
Abnormal	36	72	0	0	
Total	**50**	**100**	**50**	**100**	
Inference	Abnormal neurological examination is significantly more 36 (72%) in cases when compared to cases with p value-<0.001				

Figure :21. Shows Neurological Examination of Neonates Studied

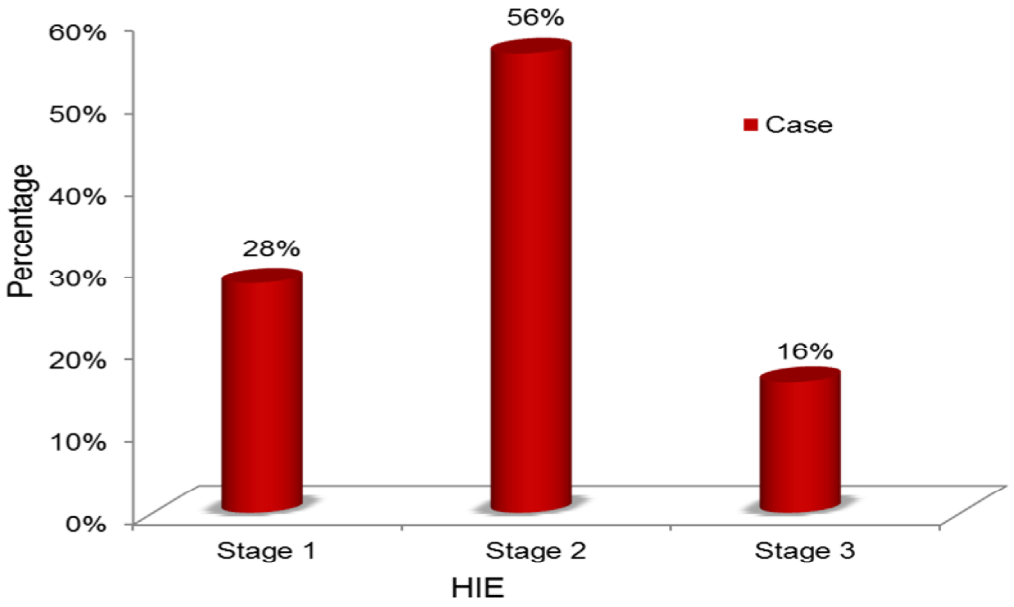


Among the 50 neonates in case group 14 (28%) had normal neurological examination with normal tone. 36 (72%) neonates had tone abnormalities.Out of 36 neonates 28(56%) had mild and marked hypotonia and 8(16%) were flaccid with severe hypotonia. All the 50(100%) neonates in control group had normal neurological examination. Abnormal neurological examination is significantly more(72%) in cases compared to controls with p value 0.001

Table :17. Shows Distribution of HIE Stages in Cases

HIE	Number of neonates (n=50)	%
Stage 1	14	28%
Stage 2	28	56%
Stage 3	8	16%

Figure :22. Shows Distribution of HIE Stages in Cases

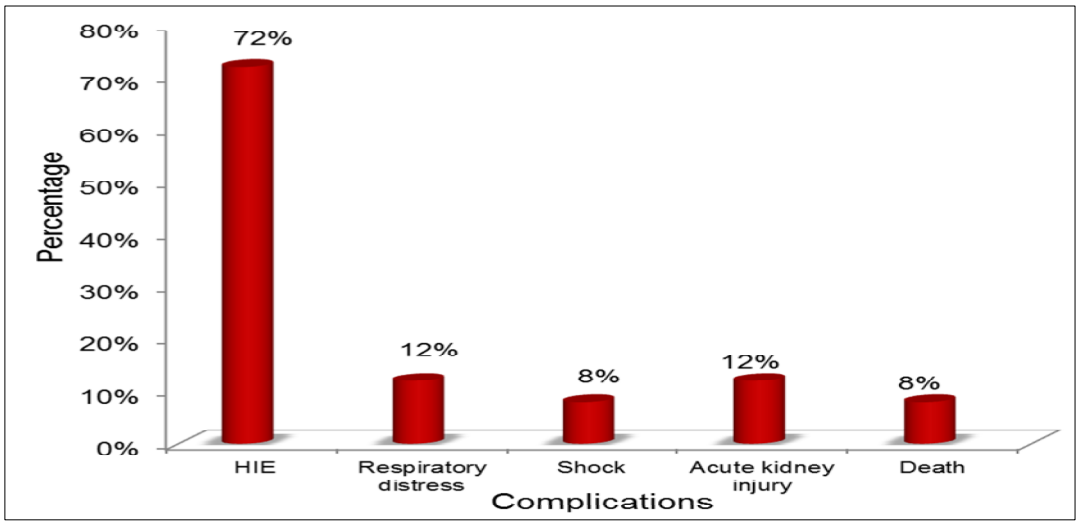


Among the 50 cases studied in the case group 14 (28%) had stage 1 HIE, 28 (56%) had stage 2 HIE and 8 (16%) had stage3 HIE during the course in NICU. Out of 8 stage 3 HIE cases 4 cases died.

Table :18. Shows Complications in Cases

Complications	No of cases(n=50)	%
HIE	36	72
Respiratory distress	6	12
Shock	4	8
Acute kidney injury	6	12
Death	4	8
Total	**50**	**100**

Figure :23. Shows Complications in Cases



Among the 50 cases in case group 36(72%) had stage2 and stage3 HIE, 6 (12%) had respiratory distress, 4(8%) had shock requiring inotrope support, 6 had acute kidney injury. 4(8%) neonates died. Of the 4 neonates died all had stage 3 HIE and required ventilatory support.

Table:19. Shows Comparison of Cut-off Levels of CK-MB and LDH In Cases and Controls

CK-MB	Case	Control	P – value
Mean ± SD	128.1 ± 107.8	24.1 ± 5.58	< 0.001**
Median (Range)	85.5 (24 - 427)	24 (14 - 36)	
LDH			
Mean ± SD	1124.2 ± 509.2	180.56 ± 38.5	< 0.001**
Median (Range)	991.5 (363 - 2612)	174 (118 - 252)	

Figure: 24. Shows Comparison of Mean CK-MB Levels in Cases and Control

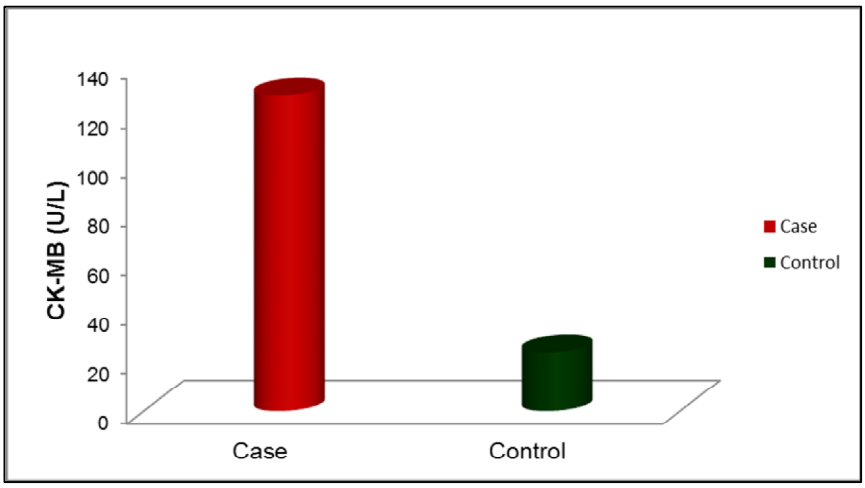
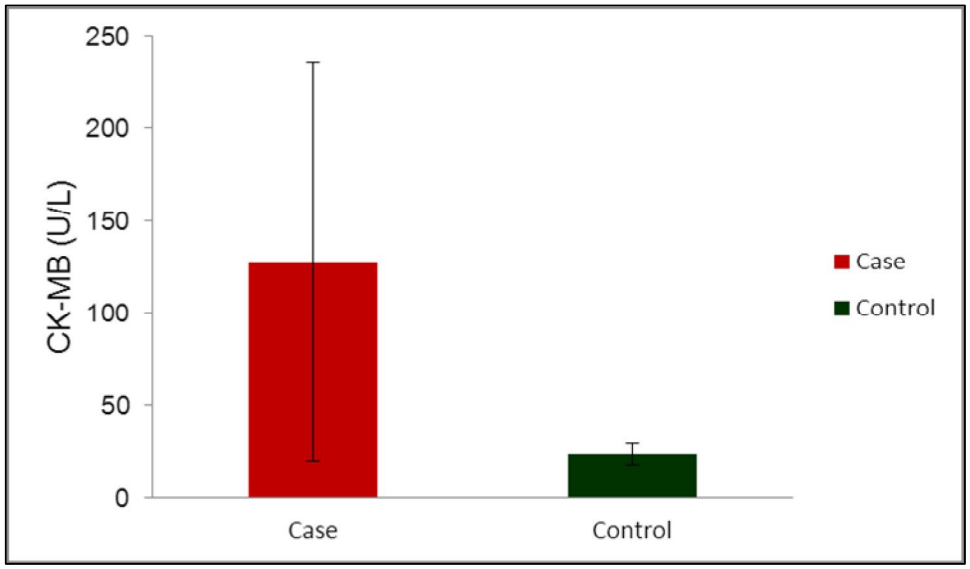


Figure: 25.Shows Comparison of Mean CK-MB Levels in Cases and Control



The mean levels of CK-MB at 8±2 hours was 128.1±107.8 U/L in case group and 24.1 ± 5.58 U/L in the control group. The mean value of CK-MB is statistically higher in case group compared to control group with P value<0.001

Figure: 26. Shows Comparison of Mean LDH Levels in Cases and controls

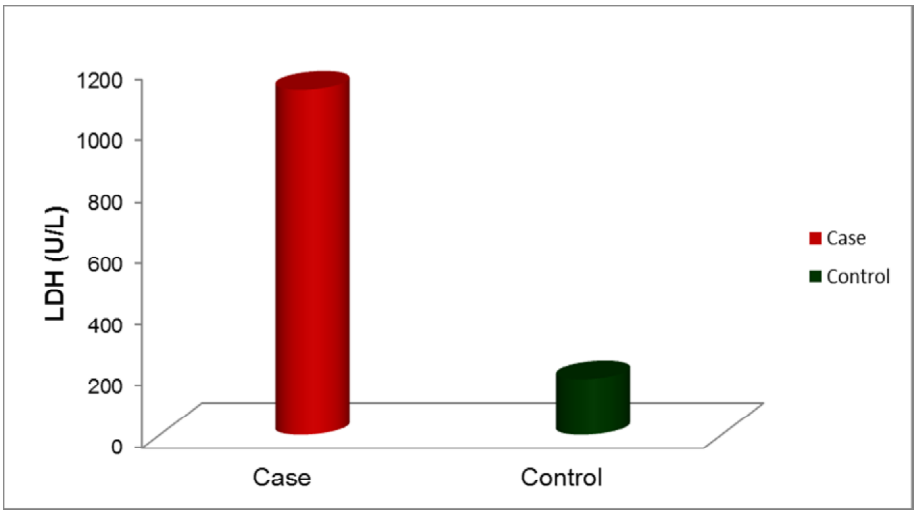
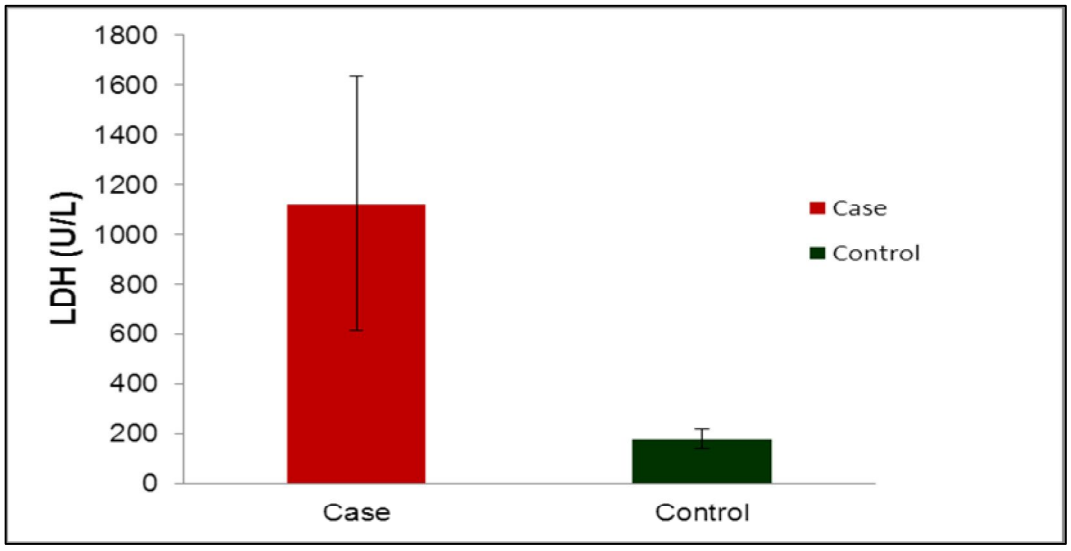


Figure: 27. Shows comparison of mean LDH levels in cases and controls



The mean LDH levels at 72±2 hours was1124.2 ± 509.2 U/L in case aroup and180.56 ± 38.5 U/L in the control group. The mean value is statistically higher in case group compared to control group with P value<0.001.

Table: 20. Shows Sensitivity, Specificity and Predictive Values of CK-MB and LDH

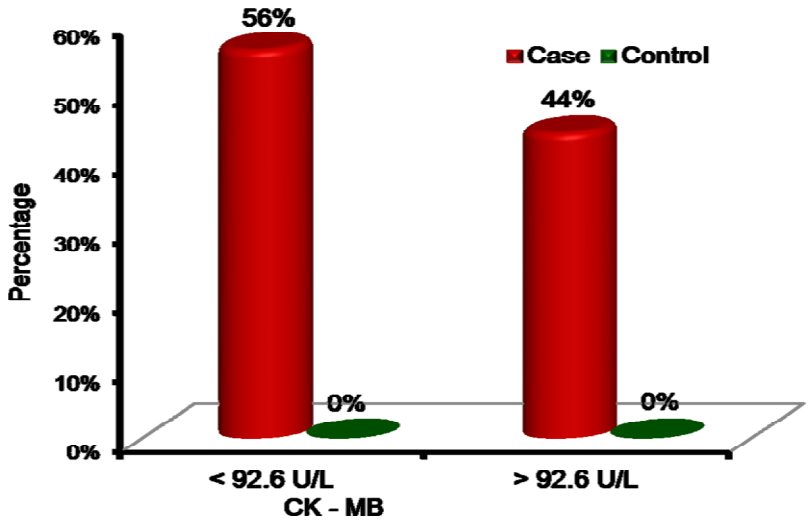
	Cut-off	Sensitivity	Specificity	PPV	NPV	Results
CK-MB (U/L) (at 8 hrs)	> 92.6	52%	100%	100%	58.14%	Excellent
LDH (U/L) (at 72 hrs)	>580	84%	100%	100%	64.94%	Excellent

The cut-off value of CK-MB >92.6 U/L has 52% sensitivity with a specificity of 100%. CK-MB has a positive predictive value of 100% with a negative predictive value of 58.14%. The cut off value of LDH >580 U/L has 84% sensitivity with a specificity of 100%. LDH has a positive predictive value of100% with a negative predictive value of 64.94%.The diagnostic performance of LDH is better than CK-MB.

Table :21. Shows comparison of cut-off levels of CK-MB and LDH in cases and controls

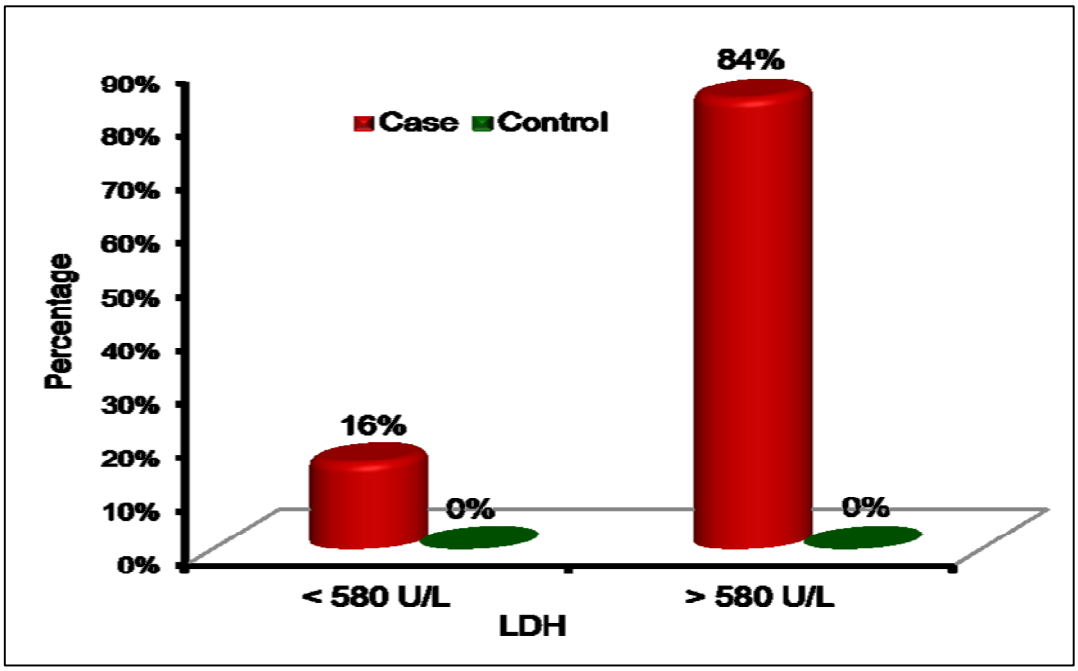
	Cases (n=50)	Control (n = 50)	P value
CK-MB (Cut-off 92.6 U/L)	50	50	
< 92.6 U/L	28 (56%)	50 (100 %)	< 0.001**
> 92.6 U/L	22 (44%)		
LDH (Cut-off 580 U/L)	50	50	
< 580 U/L	8 (16 %)	50 (100 %)	<0.001**
> 580 U/L	42 (84 %)		

Figure:28. Shows comparison of cut –off levels of CK-MB in cases and Controls



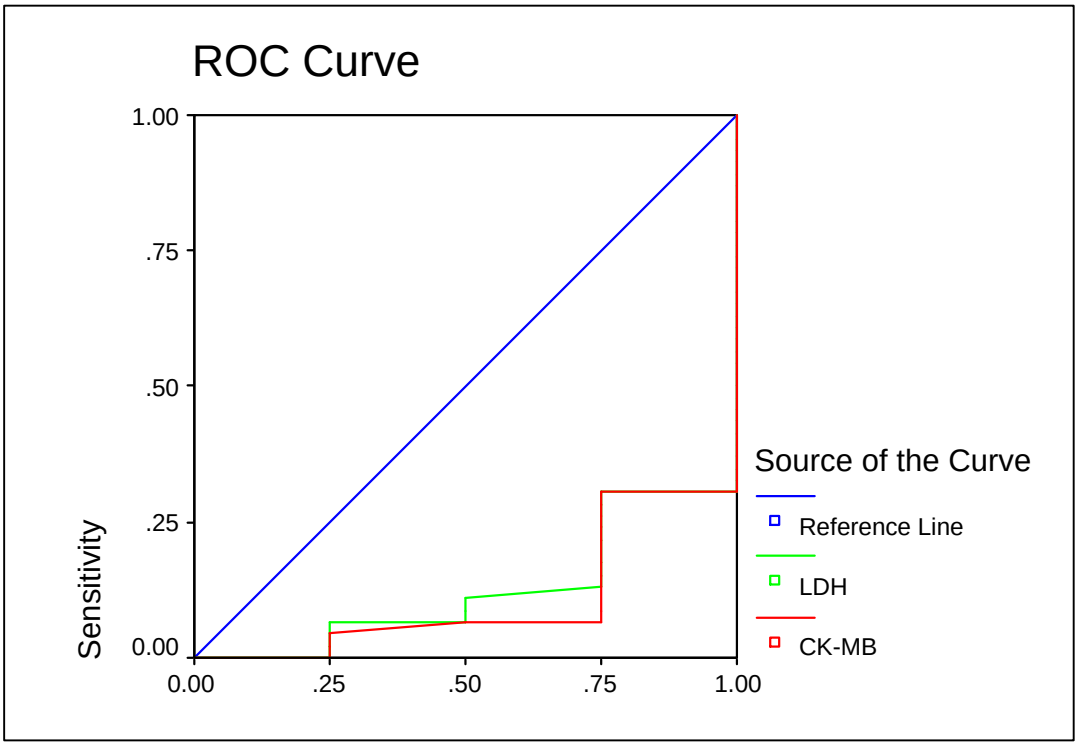
Among the 50 neonates in the case group, 28 (56%) had CK-MB levels < 92.6 U/L and 22 (44%) had CK-MB levels >92.6 U/L. Among the 50 neonates in the control group none of the cases had CK – MB levels > 92.6 U/L. The number of neonates with CK-MB > 92.6U/L is significantly more in cases when compared to controls with p value <0.001.

Figure:29. Shows comparison of cut –off levels of LDH in cases and Controls



Among the 50 neonates in the case group, 8 (16%) had LDH levels < 580 U/L and 42 (84%) had LDH levels >580 U/L. Among the 50 neonates in the control group none of the cases had LDH levels > 580 U/L. The number of neonates with LDH > 580 U/L is significantly more in cases when compared to controls with p value <0.001.

Figure:30. Shows Comparison of Receiver Operator Characteristics (ROC) Curves of CK-MB and LDH



We generated ROC (receiver operator characteristics) curves for CK-MB and LDH. The LDH had the highest ability to discriminate between cases and controls. The area under the ROC was highest for LDH (0.122) followed by CK-MB (0.106). LDH is having more diagnostic value than CK-MB, but both are excellent tests to differentiate asphyxiated and non asphyxiated newborns.

All newborns (8) with HIE stage III had significant elevation of CK-MB and LDH levels. Among 8 newborns, 4 babies died and 4 babies are survived. Survivors had abnormal neurological examination on discharge. These high risk babies were followed up and their parents were advised to attend pediatric OPD for frequent neurological examinations, developmental assessment and early stimulation.

Table 22 shows Median (Range) CK-MB and LDH levels in HIE group and non HIE group

	HIE (stage 2 and 3) Group (no=36)	Non HIE Group (no=14)	Moderate to Severe Asphyxia at 5 Min(no=24)
CK-MB level Cut off value-92.6 U/L	73-427 U/L	25-91 U/L	122-427 U/L
LDH Level Cut off Value -580 U/L	675-2612 U/L	363-667 U/L	738-2612 U/L

Median CK-MB and LDH levels are significantly higher in HIE group(72%) and moderate to severe asphyxiated neonates (58%) at 5 minute. Serum CK-MB levels correlates with immediate outcome and LDH levels correlates with long term neurological outcome.

DISCUSSION

Birth asphyxia is a common neonatal problem and contribute to significant perinatal morbidity and mortality. Birth asphyxia is a important preventable cause of cerebral injuy occuring in the newborn period. Prediction of outcome of birth asphyxia is important[81]. Apgar score has a limited role in predicting outcome such as HIE and the long term sequelae.Various studies have shown that cerebral function monitoring such as EEG, ultrasonogram of cranium, doppler measurement of cerebral blood flow and neuroimaging such as magnetic resonance imaging are useful in predicting outcome. Estimation of neurophysiological markers such as brain specific LDH isomer, CK-BB, glutamate and neurone specific enolase in cerebrospinal fluid are also useful in predicting immediate dysfunction and the long term outcome[81,82,83]. But none of the facilities are routinely available except some tertiary hospitals[84]. The signs of birth asphyxial injury are overlap with other illnesses.In the absence of perinatal records it is difficult to make a diagnosis of birth asphyxia. The current problem is inability to differentiate true positive birth asphyxiated or compromised neonate from false positive neonate. So far several studies have been conducted to evaluate better markers to differentiate an asphyxiated from non asphyxiated newborn.

In the present study an attempt has been made to ascertain wheather serum CK-MB and LDH level can distinguish an asphyxiated from non asphyxiated term newborn and whether these enzyme levels correlate with severity of birth asphyxia.These studies are routinely available in many centres and a comparative study was done to establish the usefulness of these enzyme based on previous studies.

Table :23. Comparative Study of Baseline Characteristics of Cases and Controls

Characteristics		Reddy S et al[25]		Present study	
		Cases (n=25)	Controls (n=20)	Cases (n=50)	Controls (n=50)
Sex	Male	64%	90%	54%	56%
	Female	36%	10%	46%	44%
Maternal History	Primi	525	35%	34%	29%
	Multi	48%	65%	16%	21%
Mode of Delivery	Normal	42%	85%	28%	44%
	Instrumental			10%	0
	Cesarean	58%	15%	62%	56%
Non reassuring NST		92%	0%	74%	0
MSAF		85%	0%	66%	0

In the present study, gender distribution of neonates is stastistically similar and the results are comparable to **Reddy S et al**[25]. The incidence of birth asphyxia is more in male new borns in both the studies.

There was no significant association between birth asphyxia and the parity of mothers which is comparable to **Reddy S et al**.[25]

Incidence of cesarean section and instrumental delivery were more in cases compared to controls with p value < 0.001 in the present study.This is comparable to Reddy S et al in which significantly more cases compared to controls delivered by emergengy cesarean section.(14(58%) Vs. 3(15); p=0.003. Evidence of fetal distress in the form of non reassuring NST was seen in 74% of cases in the present study compared to 92% m Reddy S et al [30] and MSAF was seen in 66% of the cases in the present study and in only 8% in **Reddy S et al**[25]. This difference could be attributed to differences in the inclusion criteria for the cases.

Table:24. Comparative studies of complications in cases

Complications		**Reddy S et al[25] (n=25)**	**Rajakumar PS et al[26] (n=30)**	**Karunatilaka DH et al[23] (n=3 5)**	**Present study (n=50)**
HIE	Total	-	100%	25.71%	100%
	Mild	-	26.67%	14.28%	28%
	Moderate	-	60%	8.58%	56%
	Severe	0%	13.33%	2.85%	16%
Hypotonia		68%	73.33%	-	72%
Cardiogenic shock		16%	16.7%	-	8%
CCF		-	36.7%	-	-
Respiratory distress		-	66.7%	-	12%
Death		-	16.7%	-	8%

In the present study, the incidence of mild HIE is 28% and 56% of cases had moderate HIE and 8% of cases had severe HIE. The incidence in the present study is slightly higher when compared to **Karunatilaka DH et al**[23] in which 25.71% of the cases had HIE. The differences in the incidence of HIE and involvement of other organ systems in birth asphyxia in different studies could be attributable to major differences in the inclusion criteria for the cases, grading system used, non reassuring fetal heart rate patterns, meconium-stained amniotic fluid, a low Apgar score at 1 minute and mild to moderate acidemia in predicting the extent and severity of hypoxic-ischemic injury to brain and other organs, initiation and effectiveness of resuscitative measures at birth, level of neonatal intensive care, post asphyxial monitoring and management of the asphyxiated newborns.

However the incidence of shock in the present study is closer to Reddy S et al [30] and **Rajakumar PS et al**[26]. In the present study, of the 4 asphyxiated neonates who had died had shock requiring ionotropic support. This is comparable to **Rajakumar PS et al**[26] in which all the 5 (16.7%) cases with cardiogenic shock had died. The 4 deaths in the present study were attributable to cardiac problems (shock), neurological problems (severe HIE) and renal problems (ARF).

One of the limitations of the present study was the inability to document umbilical arterial pH in cases though the neonates had met the inclusion criteria to be enrolled as asphyxiated neonates.

Table: 25. Comparative study of CK-MB level cut-off of 92.6 U/L in cases and controls

	Cases (U/L)		Controls (U/L)		P Value
	<92.6	**>92.6**	**<92.6**	**>92.6**	
Reddy S et al (CK-MB at 8 hours)	64%	36%	100%	0%	0.006
Present study (CK-MB at 8 hours)	56%	44%	100%	0%	<0.001

In the present study the number of neonates with CK-MB levels >92.6 U/L is significantly more in cases when compared to controls with P value<0.001. 44% of the cases in the present study had CK-MB levels >92.6 U/L. This is comparable to Reddy S et al m which 36% of cases had CK-MB levels >92.6 U/L (P=0.006).

In the present study the mean serum CK-MB levels were significantly higher in cases compared to controls with P<0.001 which is comparable to **Reddy S et al** [25] and **Rajakumar PS et al**[26].

Table: 26. Comparative studies of diagnostic performance of CK-MB

	Sensitivity	Specificity	PPV	NPV
Reddy S et al (CK-MB at 8 hours)	36%	100%	100%	52%
Rajakumar PS et al (CK-MB at 6 hours)	56.5%	75.7%	-	-
Present study (CK-MB at 8 hours)	52%	100%	100%	58.14%

In the present study, the sensitivity, specificity, PPV and NPV of CK-MB at 8 hours were 52%, 100%, 100% and 58.14% respectively, which is comparable to **Reddy S et al**[25].

Table: 27. Comparative study of LDH level cut-off of 580 U/L in cases and controls

	Cases (U/L)		Controls (U/L)		P value
	<580	>580	<580	>580	
Reddy S et al (LDH at 72 hours)	0%	100%	90%	10%	<0.001
Present study (LDH at 72 hours)	16%	84%	100%	-	<0.001

In the present study 84% of the cases had LDH levels >580 U/L. This is lower when compared to **Reddy S et al**[25] in which 100% of cases had LDH levels >580U/L. One of the limitation of the study by Reddy S et al was not excluding babies with hemolytic disease in the case group which could explain more number of cases having LDH levels >580 U/L.

In both the studies, the number of neonates with LDH levels >580 U/L is significantly more in cases when compared to controls. This is statistically significant in both the studies with P<0.001.

In the present study the mean LDH levels in case group were significantly higher when compared to control group with P<0.001 which is comparable to **Reddy S et al**[25].

Table: 28. Comparative studies of diagnostic performance of LDH

	Sensitivity	**Specificity**	**PPV**	**NPV**
Reddy S et al (LDH at 72 hr)	100%	89%	92%	100%
Karlsson M et al	100%	97%	87%	100%
Present study (LDH at 72 hr)	84%	100%	100%	64.94%

In the present study, the sensitivity, specificity, PPV and NPV of LDH at 72 hours were 84%, 100%%, 100% and 64.94% respectively. The Specificity and PPV are comparable to **Reddy S et al**[25] and **Karlsson M et al**[27].

SUMMARY

- This is a prospective study conducted over a period of 1 year from June 2013 to May 2014 in the department of Pediatrics, government Mohan Kumaramangalam Medical College & hospital, Salem.
- The blood samples from 50 neonates comprising the cases and 50 neonates comprising the controls constituted the material for the study.
- Incidence of cesarean section and instrumental delivery are significantly more in case group 36 (72%) compared to control group 22 (44%).
- 13 (26%) had Reassuring NST and 37 (72%) had Non Reassuring NST suggestive of fetal distress in the case group.
- 33 (66%) had Thick MSAF and 17 (34%) had clear liquor in case group.
- All the 50 (100%) neonates in the case group had an Apgar score <7 at 1min. 14(28%) cases had 0-3 (severe birth asphyxia) and 36 (72%) had 4-6 (moderate birth asphyxia). Among the 50 neonates in case group 26(52%) neonates had an apgar score of ≥ 7 at 5 min

following resuscitation at birth. 24(48%) had an apgar score <7 at 5 min even with resuscitation.

- 14 (28%) had normal neurological examination with normal tone. 36 (72%) had hypotonia on neurological examination.
- Among the case group 14 (28%) had stage I HIE, 28 (56%) had stage II HIE and 8 (16%) had severe HIE during the course in NICU.
- In the case group 36 (72%) had HIE stage II and III, 6 (12%) neonates had respiratory distress. 4 (8%) neonates had shock requiring inotrope support, 6 (12%) had acute kidney injury .4 (8%) neonates died. Of the 4 neonates died had HIE stage III and required ventilatory support and inotrope support.
- 28 (56%) had CK-MB levels <92.6 U/L and 22 (44%) had CK-MB levels >92.6 U/L in the case group. None of the neonate in control group had CK-MB levels >92.6 U/L.
- 8 (16%) had LDH levels <580 U/L and 42 (84%) had LDH levels >580 U/L in the case group. None of the neonate in control group had LDH levels >580 U/L.
- The cut-off CK-MB value of >92.6 U/L has 52% sensitivity with a specificity of 100%. CK-MB has a positive predictive value of 100% with a negative predictive value of 58.14%.

- The cut-off LDH value of >580 U/L has 84% sensitivity with a specificity of 100%. LDH has a positive predictive value of 100% with a negative predictive value of 68.94%.
- 4 babies died as a result of birth asphyxia had marked elevation of CK-MB and LDH when compared to other cases and clinically had HIE stage 3. This indicates both CK-MB and LDH levels could be able to correlate with severity of HIE.
- LDH is having more diagnostic value than CK-MB, but both are excellent tests to differentiate asphyxiated and non-asphyxiated neonates.
- CK-MB and LDH are biochemical markers more useful to correlate with severity of HIE.

CONCLUSION

- Birth asphyxia is a common neonatal problem and contributes to significant neonatal morbidity and mortality.
- The signs of asphyxial injury are often overlap with other illnesses. In the absence of perinatal records, it is difficult to make a diagnosis of Birth asphyxia.
- Prediction of outcome of Birth asphyxia is important but formidable.
- There is a need to identify neonates born with birth asphyxia who will be at high risk for developing HIE and early neonatal death as a consequence of Birth asphyxia.
- Leakage of enzymes like CK-MB and LDH signalling MOD is seen together with HIE after birth asphyxia. CK-MB and LDH estimation is available in most centres and are comparatively cheaper tests when compared to neuroimaging studies.
- LDH is having more diagnostic value than CK-MB with more area under ROC value, but both are excellent tests to differentiate asphyxiated and non-asphyxiated term neonates.
- Estimation of serum levels of CK-MB and LDH can help distinguish an asphyxiated from a non-asphyxiated term neonate with reasonable degree of accuracy and could be able to correlate with severity of birth asphyxia.

- The results of the present study could be of utility to pediatricians in referral hospitals, who receive sick neonates, whose birth records are not well recorded. CK-MB and LDH could be used to make a diagnosis of birth asphyxia in such cases in correlation with history and clinical features in the neonate.
- This study also helps in effective and aggressive management of severe birth asphyxia in the immediate neonatal period thereby reducing the mortality. To have regular follow-up of asphyxiated babies for early diagnosis and early intervention of long term neurological sequelae for better outcome.
- All survivors of moderate to severe hypoxic ischemic encephalopathy require comprehensive high risk medical and developmental follow-up.
- Early identification of neurodevelopmental problems allows prompt referral for developmental, rehabilitative, neurologic care and early intervention services so that the best possible outcome can be achieved.
- This study help us to identify high risk babies at the earliest age and for future follow up.

BIBLIOGRAPHY

1. NNPD network. National Neonatal Perinatal Database-report for the year 2002-2003. NNF NNPD network. New Delhi: 2005.
2. Lawn JE, Cousens S, Zupan J. Lancet Neonatal Survival Steering Team. 4 million neonatal deaths: When? Where? Why? Lancet 2005; 365 (9462):891-900.
3. Perlman JM, Tack ED, Martin T, Shackelford G, Amon E. Acute systemic organ injury in term infants after asphyxia. Am J Dis Child 1989; 143:617-620.
4. Addock LM, Papile L. Perinatal asphyxia. In: Cloherty JP, Eichenvrald EC, Stark AR, editors. Manual of neonatal care. 6th edition Philadelphia: Lippincott Williams and Wilkins, a Wolters Khivrar business, 2008: p.518-528.
5. Karlsson M, Blennow M, Nemeth A, Winbladh B. Dynamics of hepatic enzyme activity following birth asphyxia. Acta Paediatrica 2006,95:1405-1411.
6. Lackmann GM, Tollner U, Mader R. Serum enzyme activities in full-term asphyxiated and healthy newborns: enzyme kinetics during the first 144 hours of life. Enzyme & Protein 1993,47:160-172.
7. Zanardo V, Bondio M, Perini G, Temporin GF. Serum glutamic-oxaloacetic transaminase and glutamic-pyruvic transaminase activity in premature and full-term asphyxiated newborns. Biology of the Neonate 1985, 47:61-69.
8. George F. Smith, MD. Historical Review and Recent Advances in Neonatal and Perinatal Medicine. Recent Advances in Neonatal and Perinatal Medicine 2006, 11:1-8.
9. Indian Institute of Population Studies. National Family Health Survey (NFHS-2) 1998-99. Mumbai: 2000.

10. Little WJ. On the Influence of Abnormal Parturition, Difficult Labours, Premature Birth and Asphyxia Neonatorum on the Mental and Physical Condition of the Child, Especially in Relation to Deformity. Trans. Obstet. Soc. London 1861, 3:293.
11. Eastman NJ. Foetal Blood Studies 1. The Oxygen Relationship of Umbilical Cord Blood and Birth. Bull. J. Hop. Hosp. 1930; 47,4:221.
12. Eastman NJ, McLane CM. Foetal Blood Studies II. The Lactic Acid Content of Umbilical Cord Blood Under Various Conditions. Bull. J. Hop. Hosp. 1931, 48:26.
13. Apgar V. A proposal for a new method of evaluation of the neWoorn infant Curr Res Anesth Analg 1953; 32:260-267.
14. James FS, Weisbrot IM, Prince CE. The Acid Base Status of Human Infants in Relation to Birth Asphyxia and the Onset of Respiration. J. Pediatr. 1958; 52:379.
15. Windle WF. Asphyxia Neonatorum. Springfield, Illinois: Charles C. Thomas, 1950.
16. Myers RE. Experimental Models of Perinatal Brain Damage: Relevance to Human Pathology. In Gluck, L. (ed.): Intrauterine Asphyxia and the Developing Fetal Brain. Chicago: Year Book Medical Publishers, Inc., 1977.
17. Primhak RA, Jedeikin R, Ellis G, Makela SK,Gillan JE, Swyer PR, et al. Myocardial ischaemia in asphyxia neonatorum Electrocardiographic, enzymatic and histological correlations. Acta Pediatr Scand 1985; 74: 595-600.
18. Sanchez-Nava J, Gonzalez-Carreno S, Hernandez-Martinez JA, Renteria MA. Increase in glutamic-oxaloacetic and glutamic-pyruvic transaminases and lactic dehydrogenase as a diagnostic aid in perinatal asphyxia. Bol Med Hosp Infant Mex 1990; 47: 372-375.

19. Omokhodion SI, Losekoot TG, Jaiyesimi F. Serum creatine kinase and creatine kinase-MB isoenzyme activities in perinatally asphyxiated newborns.Eur Heart J. 1991 Sep; 12(9): 930-984.
20. Fonseca E, Garcia-Alonso A, Zarate A, Ochoa R, Gal van RE, Jimenez-Soli s G. Elevation of activity of creatine phosphokinase (CK) and its isoenzymes in the newborn is associated with fetal asphyxia and risk at birth. Clin Biochem 1995 Feb;23(l):91-5.
21. Lackmann GM. Influence of neonatal idiopathic respiratory distress syndrome on serum enzyme activities in premature healthy and asphyxiated newborns. Am J Perinatal 1996; 13: 329-334.
22. Barberi I, Calabro MP, Cordaro S, Gitto E, Sottile A, Prudente D, et al. Myocardial ischemia in neonates with perinatal asphyxia. Electrocardiographic, echocardiographic and enzymatic correlations. Eur J Pediatr 1999; 158: 742-747.
23. Karunatilaka DH, Amaratunga GWDS, Perera KDNI, Caldera V. Serum creatine kinase and lactic dehydrogenase levels as useful markers of immediate and long-term outcome of perinatal asphyxia. Sri Lanka Journal of Child Health, 2000; 29: 49-52.
24. Boo NY, Hafidz H, Nawawi HM, Cheah FC, Fadzil YJ, Abdul-Aziz BB, et al. Comparison of serum cardiac troponin T and creatine kinase MB isoenzyme mass concentrations in asphyxiated term infants during the first 48 h of life. J Paediatr Child Health. 2005 Jul;41(7):331-7.
25. Reddy S, Dutta S, Narang A. Evaluation of Lactate Dehydrogenase, Creatine Kinase and Hepatic Enzymes for the Retrospective Diagnosis of Perinatal Asphyxia Among Sick Neonates. Indian Pediatrics February 17, 2008; 45: 144-147.

26. Rajakumar PS,Vishnu Bhat B, Sridhar MG, Balachander J, Konar BC, Narayanan P, et al. Cardiac Enzyme Levels in Myocardial Dysfunction in Newborns with Perinatal Asphyxia. Indian Journal of Pediatrics December, 2008;75:1223-1225.
27. Karlsson M, Wiberg-Itzel E, Chakkarapani E, Blennow M, Winbladh B, Thoresen M. Lactate dehydrogenase predicts hypoxic ischaemic encephalopathy in newborn infants: a preliminary study .A eta Paediatrica August 2010; 99(3): 1139-1144.
28. Agarwal R, Jain A, Deorati AK, Paul VK. Post-resuscitation management of asphyxiated neonates. Indian Journal of Pediatrics February,2008; 75:175-180.
29. American Academy of Pediatrics, Committee on Fetus and Newborn: Use and abuse of the Apgar score. Pediatrics 1986,78:1148.
30. Tooly J. Perinatal asphyxia and Hypoxic-ischemic encephalopathy. Perinatal /Neonatal medicine. In: Mclntosh N, Helms P, Smyth R, Logan S, editors. Forfar and Arneil's textbook of Pediatrics. 7th edition Philadelphia: Churchill Livingstone, an imprint of El sevier Ltd; 2003. p. 204-207.
31. Perinatal Asphyxia In: Guha DK, Guha R, Dore P, editors. Neonatal Asphyxia, Resuscitation & Beyond.2nd edition. New Delhi: Jaypee Brothers Medical Publishers(P) Ltd; 2008. p.7-14.
32. Meharban Singh. Introduction to care of newborn babies. Care of the newborn seventh edition. 2010;1: 1-12.
33. ACOG, ACOG Committee Opinion. Number 326. Inappropriate use of the terms fetal distress and birth asphyxia. Obstet Gynecol 2005; 106:1469-1470.
34. Phelan JP, Martin GI, Korst LM. Birth asphyxia and cerebral palsy. Clin Perinatol 2005; 32:61-76.VI.

35. John P.Clohety, Eric C. Eichenwald, Anne. R.Hansen, Ann R. Stark. Perinatal Asphyxia and Hypoxic-Ischemic Encephalopathy. Manual of Neonatal Care. 2012. 55: 711-728.
36. Curtis PD, Matthews TG, Clarke TA, Darling M, Crowley P, Griffin E, et al. Neonatal seizures: The Dublin Collaborative Study. Arch Dis Child 1988, 53:1065-1058.
37. Nelson KB, Ellenberg JH. Obstetric complications as risk factors for cerebral palsy or seizure disorders. JAMA 1984, 251:1843-1848.
38. Richey SD, Ramin SM, Bawdon RE, Roberts SW, Dax J, Roberts J, et al. Markers of acute and chronic asphyxia in infants with meconium-stained amniotic fluid. American Journal of Obstetrics and Gynecology. 1995, 172: 1212-1215.
39. John P.Clohety, Eric C. Eichenwald, Anne. R.Hansen, Ann R. Stark. Fetal Assessment and Prenatal Diagnosis. Manual of Neonatal Care. 2012. 1: 1-10.
40. Dildy GA. Intrapartum Assessment of the Fetus: Historical and Evidence -Based Practice. Obstet Gynecol Clin N Am 2005; 32:255-271
41. Garite TJ. Intrapartum fetal evaluation. Gabbe: Obstetrics - Normal an Problem Pregnancies, 4th ed. (2002)
42. Meharban Singh. Preventive Neonatology. Care of the newborn seventh edition. 2010;3: 34-57.
43. John P.Clohety, Eric C. Eichenwald, Anne. R.Hansen, Ann R. Stark. Fetal Assessment and Prenatal Diagnosis. Manual of Neonatal Care. 2012. 1: 1-10.
44. Meharban Singh. Preventive Neonatology. Care of the newborn seventh edition. 2010;3: 34-57.
45. John P.Clohety, Eric C. Eichenwald, Anne. R.Hansen, Ann R. Stark. Perinatal Asphyxia and Hypoxic-Ischemic Encephalopathy. Manual of Neonatal Care. 2012. 55: 711-728.

46. Stoll BJ. Routine delivery room care. The Newborn The fetus and the Neonatal Infant. In: Kliegnan RM Behrman RE, Jenson HB, Stanton BF, eds. Nelson textbook of Pediatrics. 18th edition. Philadelphia: Saunders ,2 007. p. 679-680.
47. Meharban Singh. Preventive Neonatology. Care of the baby in the labor room seventh edition. 2010;6: 85-107.
48. American College of Obstetrics and Gynecology, Task Force on Neonatal Encephalopathy and Cerebral Palsy; American Academy of Pediatrics. *Neonatal Encephalopathy and Cerebral Palsy: Defining the Pathogenesis and Pathophysiology*. Washington, DC: American College of Obstetricians and Gynecologists; 2003.
49. Papile LA. The Apgar score in the 21st century. *N Engl J Med.*2001;344:519–520
50. Casey BM, McIntire DD, Leveno KJ. The continuing value of the Apgar score for the assessment of the newborn infants. *N Engl J Med.* 2001; 344:467–471.
51. Robert. M.Kliegman, Richard E.Behrman, Hal.B.Jenson, Bonita F.Stanton. The Fetus and the neonatal infant. Nelson text book of pediatrics. 2008, 94:675-683.
52. American College of Obstetricians and Gynecologists: Use and abuse of the Apgar score. Committee Opinion, No. 174, July 1996.
53. Robert. M.Kliegman, Richard E.Behrman, Hal.B.Jenson, Bonita F.Stanton. The Fetus and the neonatal infant. Nelson text book of pediatrics. 2008, 99:713-722.
54. American College of Obstetricians and Gynecologists: Inappropriate use of the terms fetal distress and birth asphyxia. Committee Opinion No. 303, October 2004.
55. Kumar V, Abbas AK, Fausto N, Mitchel R. Robbins Basic Pathology. 8th edition. Philadelphia: Saunders/Elsevier, 2008.
56. Meharban Singh. Preventive Neonatology. Care of the baby in the labor room seventh edition. 2010;6: 85-107.

57. Volpe JJ. Hypoac-ischernic encephalopathy: neuropathology and pathogenesis. In: Volpe JJ. Neurology of the newborn, 3rd edn, WB Saunders Philadelphia 1995, pp 279-313.
58. Johnston MV, Trescher WH, Ishida A,et al, Neurobiology of hypoxic ischemic injury in the developing brain.ped res 2001;49(6):735-741.
59. Sarnat HB, Sarnat MS. Neonatal encephalopathy following fetal distress: A clinical and electro encephalographies study. Arch Neurol 1976,33:696.
60. Levene M, Evans D. Hypoxic-ischaemic brain injury. Neurological problems in the newborn. In:Rennie JM, editor. Roberton's textbook of Neonatology. 4th edition. Philadelphia: Elsevier,2005. p. 1128-1148.
61. Myers RE. Four patterns of perinatal brain damage and their conditions of occurrence in primates. Advances in Neurology 1975, 10: 223-234.
62. Barkovich AJ, Westmark K, Partridge C, et al. Perinatal asphyxia. MR findings in the first 10 days. AJNR Am J Neuroradiol 1995; 16(3): 427-438.
63. Mckinstry RC, Miller JH, Snyder AZ, et al., A prospective, longitudinal diffusion imaging study of brain injury in newborns. Neurology. 2002; 59(6):824-833.
64. Soul JS, Robertson RL, Tzika AA, et al., Time Course of changes in diffusion weighted magnetic resonance imaging in a case of neonatal encephalopathy with defined onset and duration of hypoxia ischemic insult pediatrics. 2001;108(5): 1211-1214.
65. Shah P, Riphagen S, B eyene J, Perlman M. Multi organ dysfunction in infants with post-asphyxial hypoxic-ischaemic encephalopathy. Archives of Disease in Childhood Fetal & Neonatal Edition 2004; 39:F152-155.
66. Hankins GDV, Koen S, Gei AF, Lopez SM, Van Hook JW, Anderson GD. Neonatal organ system injury in acute birth asphyxia sufficient to result in neonatal encephalopathy. Obstetrics & Gynecology 2002, 99:688-691.

67. Levene M. Perinatal asphyxia and Hypoxic-ischemic encephalopathy. In: Mclntosh N, Helms P, Smyth R, editors. Forfar and Ameil's textbook of Pediatrics. 6th edition. Philadelphia: Churchill Livingstone, an imprint of Elsevier Ltd, 2003. p.197-204.
68. Robert DS, Haycock GB, Dalton RN, Turner C, Tomlinson P, StimmlerL, et al. Prediction of acute renal failure after birth asphyxia. Arch Dis Child 1990; 65:1021-1023.
69. Arkhngel'skii AV, Masliakova GN. Frequency and morphology of DIC syndrome in children in early neonatal period. Arkh Patol. 1996; 58: 61-63.
70. Southern EM, Fetal Anoxia and it possible relation to change in prenatal fetal ECG. AM J obster Gynecol 1957; 73:233-247.
71. Turner-Gomes SO, Izukawa T, Rowe RD. Persistence of atrioventricular valve regurgitation and electrocardiographic abnonnalities following transient myocardial ischemia of the newborn. Pediatric Cardiology 1989, 10:191-194.
72. Buciarelli RL, Nelson R, Egan EA, Eitzman V, Gesser IM. Transient tricuspid insufficiency of newborn.pediatr 1977;59;3330-3333.
73. Wallimann T, Hernmer W. Creatine kinase in non-muscle tissues and cells. Molecular and Cellular Biochemistry 1994; 133-134: 193-220.
74. Schlattner U, Tokarska-Schlattner M, Wallimann T.Mitochondrial creatine kinase in human health and disease. Biochimica et Biophysica Acta 2006, 1762(2): 164-0.
75. Pesce MA. Reference ranges for laboratory tests and procedures. In: Kliegman RM Behrman RE, Jenson HB, Stanton BF, eds. Nelson textbook of Pediatrics. 18th edition. Philadelphia: Saunders,2007. p. 2943-2954.
76. Warburton D, Singer DB, Oh W. Effects of acidosis on the activity of creatine phosphokinase and its isoenzymes in the serum of newborn infants. Pediatrics 1931; 68 : 195-197.

77. Jedeikin R, Makela SK, Shennan AT, Rowe RD, Ellis G.. Creatine kinase isoenzymes in serum from cord blood and the blood of healthy full-term infants during the first three postnatal days. Clin Chem 1932; 23: 317-322.
78. Kaplowitz H. Mechanisms of liver cell injury. Journal of Hepatology 2000, 32:39-47.
79. Gibson PR, Dudley FJ. Ischemic hepatitis: clinical features, diagnosis and prognosis. Australian & New Zealand Journal of Medicine 184, 14:822-825.
80. Lackmann GM, Tollner U. The predictive value of elevation in specific serum enzymes for subsequent development of hypoxic-ischemic encephalopathy or litraventricular hemorrhage in full-term and premature asphyxiated newborns. Neuropediatrics 1995, 26:192-198.
81. Thomberg E, Thninger K, Hagberg H, Kjellmer I. Neurone specific enolase in asphyxiated newborns: Association with encephalopathy and cerebral function monitor trace. Archives of Disease in Childhood 1995,72: 39-42.
82. Ekin P, Toet M C, Groeneedaal F, de Vries L S. Predictive value of neuroimaging, pulse Dopplerand neurophysiology in full term infants with hypoxic lschaermic encephalopathy. Archives of Disease in Childhood 1995,73: 75-80.
83. Bjerre, Hellstrom- Westas, Rosen , Swenningsen H Monitoring of cerebral function after severe asphyxia in infancy. Archives of Disease in Childhood 1983,58: 997-1002.
84. Indian Institute of Population Studies. National Family Health Survey (NFHS-2) 1998-99. Mumbai: 2000.
85. Khreisat WH, Habahbeh Z. Risk factors of birth asphyxia: A study at Prince All Ben Al-Hussein Hospital, Jordan.Pak J Med Sci January-March 2005; 21(1):30-34.

ANNEXURE

PROFORMA OF THE DISSERTATION

A STUDY TO EVALUATE THE SIGNIFICANCE OF SERUM CREATINE KINASE MUSCLE BRAIN FRACTION (CK-MB) AND LACTATE DEHYDROGENASE (LDH) IN NEONATES WITH BIRTH ASPHYXIA

Baby of	:	
Sex	:	
Time and Date of Birth	:	
Birth Weight	:	
Assessment of Gestational Age	:	

Method	**LMP / EDD**	**USG**	**New Ballard Score**
Period of gestation			

Maternal History	:	
Non Stress Test	:	Reassuring / Non Reassuring
Birth Notes	:	
Thick Meconium Stained Amniotic fluid	:	Yes / No

Apgar Score	1 Min :	5 Min :	10 Min :

Examination of the Baby	:	
Head to Foot Examination	:	
Any Obvious external congenital Abnormalities	:	
Neurological examination	:	
Other Systemic examination	:	
Course in NICU	:	
HIE Stage by Sarnat and Sarnat staging	:	

Inclusion Criteria	**Yes**	**No**
1. Gestational age ≥ 37 weeks		
2. Appropriate for gestational age		
3. The neonates will be identified to have experienced Birth asphyxia when at least 3 of the following are present		
a) Intrapartum signs of fetal distress, as indicated by non reassuring NST / or by thick meconium staining of the amniotic fluid		
b) APGAR score of < 7 at one minute of life		
c) Resuscitation with >1 minute of positive pressure ventilation before stable spontaneous respiration		
d) Hypoxic ischemic encephalopathy (HIE) as defined by Sarnat and Sarnat clinical staging		

Exclusion Criteria	Yes	No
1. Congenital malformations		
2. Maternal drug addiction		
3. Neonates born to mothers who had received magnesium sulphate within 4 hours prior to delivery or opiods (Pharmacological depression)		
4. Hemolytic disease of the newborn		

Investigations	:	
1. Peripheral smear for hemolytic picture	:	

Enzymes	Serum Levels (U/L)
1. Creatine Kinase Muscle-Brain Fraction (CK-MB)	
2. Lactate Dehydrogenase (LDH)	

KEY TO MASTER CHART

SL.NO	-	SERIAL NUMBER
B/O	-	BABY OF
IP NO	-	IN PATIENT NUMBER
MCH	-	MALE CHILD
FCH	-	FEMALE CHILD
MOD	-	MODE OF DELIVERY
MOR	-	MODE OF RESUSCITATION
MH	-	MATERNAL HISTORY
NST	-	NON STRESS TEST
HIE	-	HYPOXIC ISCHEMIC ENCEPHALOPATHY
RD	-	RESPIRATORY DISTRESS
USG	-	ULTRASONOGRAM
CBC	-	COMPLETE BLOOD COUNT
CK-MB	-	CREATINE KINASE MUSCULE BRAIN FRACTION
LDH	-	LACTATE DRHYDROGENASE
AEDs	-	ANTIEPILEPTIC DRUGS