OUTCOME OF ASPHYXIATED NEWBORN, CORRELATION WITH MATERNAL FACTORS AND TO LEVEL OF OXIDATIVE STRESS IN THE NEWBORN

Dr.PIDIKITI.LAVANYA

Dissertation submitted to

BLDE (Deemed to be University) Vijayapur, Karnataka

In partial fulfillment of the requirements for the degree of

DOCTOR OF MEDICINE

IN

PEDIATRICS

Under the guidance of

Dr.R.H.GOBBUR

PROFESSOR

DEPARTMENTOF PEDIATRICS

BLDE (Deemed to be University)

SHRIB.M.PATILMEDICALCOLLEGE

HOSPITAL & RESEARCH CENTRE, VIJAYAPUR

KARNATAKA

2020

“OUTCOME OF ASPHYXIATED NEWBORN, CORRELATION WITH MATERNAL FACTORS AND TO LEVEL OF OXIDATIVE STRESS IN THE NEWBORN”

DOCTOR IN MEDICINE IN PEDIATRICS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

AHA	AMERICAN HEART ASSOCIATION
PPV	POSITIVE PRESSURE VENTILATION
DALY	DISABILITY ADJUSTED LIFE YEARS
MDA	MALONDIALDEHYDE
HIE	HYPOXIC ISCHEMIC ENCEPHALOPATHY
MOD	MULTIORGAN DYSFUNCTION
NO	NITRIC OXIDE
nNOS	NEURONOAL NITRIC OXIDE SYNTHASE
iNOS	INDUCIBLE NITRIC OXIDE SYNTHASE
AGA	APPROPRIATE FOR GESTATIONAL AGE
SGA	SMALL FOR GESTATIONAL AGE
LGA	LARGE FOR GESTATIONAL AGE

OUTCOME OF ASPHYXIATED NEWBORN, CORRELATION WITH MATERNAL FACTORS AND TO LEVEL OF OXIDATIVE STRESS IN THE NEWBORN

INTRODUCTION

A phenomenon that affects 90% of newborns without a problem, while 10% of infants will not start breathing effectively, is the establishment of effective respiration at birth and the transformation from foetal circulation to extrauterine state, which are necessary to start and maintain in extrauterine life. After first stimulation, the majority of them start breathing; just about 3-5% need basic resuscitation, and less than 1% require advanced resuscitation.[1] According to the most recent estimates, ten million of the 136 million newborns who are born each year require assistance:

1. The American Heart Association (AHA) has released the following recommendations to help prevent asphyxia, which causes major mortality and severe morbidity: Positive-pressure ventilation (PPV) stays the most important intervention in neonatal resuscitation. While monitoring of newborn resuscitation research and practises continue to advance, it is important to focus the development of PPV skills and procedures.
2. Pulse oximetry must be used carefully and as a guide when administering more oxygen. Neonatal resuscitation continues to place a high priority on preventing hypothermia.

3. In addition to anticipation, preparation, briefing, and debriefing, team education continues to be a crucial component of newborn resuscitation. To extract a Normal Newborn, a quick, high-quality response and overall performance are essential.

This guideline affirms the previous recommendations.

Birth asphyxia, which accounts for 23% of all newborn deaths globally and ranks as the fifth-leading cause of death in children under the age of five, is a serious health concern. According to the WHO, it is one of the major contributors to the burden of disease across all age groups (measured in DALYs). An estimated 1.1 million intrapartum stillbirths are linked to birth asphyxia, which is estimated to cause 920,000 neonatal deaths annually. Among the more than a million infants who survive birth asphyxia, complications like cerebral palsy, mental retardation, and learning challenges may arise.[2,3]

Primary causes are systemic hypoxemia & reduced cerebral blood flow, both of which can occur in isolation or can be seen together.

Hypoxia causes the foetus to redistribute blood flow to essential organs initially, but if asphyxia persists, even this compensatory mechanism fails, leading to ischemic brain injury.

A disorder known as perinatal asphyxia, also referred to as hypoxic-ischemic encephalopathy, is characterised by the presence of clinical and biochemical evidence of acute or subacute brain injury as a result of asphyxia.

Many clinical conditions include oxidative stress, which is normally countered by the antioxidant system. However, because oxidative stress increases during labour, this equilibrium is not maintained. Malondialdehyde (MDA) is one of the important markers of lipid peroxidation and oxidative stress is It has been observed that the aetiology of many diseases is significantly influenced by the oxidative stress brought on by increased free radical production and decreased antioxidant levels in cells or tissues like in birth asphyxia and hypoxic-ischemic encephalopathy.

NEED FOR THE STUDY

A newborn's life might be affected by the intrauterine period, which is a critical time. The relationship between birth-related factors (short gestational age, low APGAR score, presence of HIE), maternal-related factors (the presence of systemic disorders such as preeclampsia, and hypertension), and babies' exposure to oxidative stress is well documented.

The most frequent cause of convulsion in newborns, which is also associated with significant mortality & morbidity, is asphyxia. The degree of oxidative stress in the baby is a factor in the severity of hypoxia and its fate, although its impact is not fully understood. There is an increase in oxidative stress in LSCS compared to a normal delivery. Not much research has been done on the relationship between oxidative stress and birth asphyxia.

The need for resuscitation prior to birth might occasionally be signalled by the numerous pregnancy-related difficulties in mothers or neonatal issues. In this study, we evaluate the neonatal resuscitation process, evaluate the impact of various maternal complications on the resuscitation needed in the babies, correlate the levels of oxidative stress, and indirectly attempt to predict the prognosis, and may point to further studies to reduce adverse outcomes by treating with anti-oxidants.

Hence the need for the study to correlate maternal factors and neonatal resuscitation level, the oxidative stress level in resuscitated newborns with respect to the severity of asphyxia and its outcome.

AIM AND OBJECTIVES OF THE STUDY

1. To assess the method of resuscitation required, correlating to maternal risk factors and outcome of newborns.
2. To correlate resuscitation and outcome to the baby's oxidative stress level by using Malon-Di-Aldehyde (MDA) levels in the cord blood.

REVIEW OF LITERATURE

Asphyxia is a condition in which a subject has impaired gas exchange, which, depending on the degree and length of this disruption in gas exchange, can cause progressive hypoxia, hypercarbia, and acidosis. This condition can be defined as the term asphyxia. Birth asphyxia, also known as improper exchange of gases during the delivery period, is a condition for which there are no specific biochemical criteria. This means that before labelling a new born with "asphyxia," the greatest care must be taken. Unfortunately, this phrase is typically and wrongly associated with a poor neurodevelopmental outcome, which is more popularly recognised as cerebral palsy.

In a study, the assessment of the resuscitation process in the room and the relationship between risk factors for the mother and the newborn were evaluated, and they were found to be positively correlated with the amount of resuscitation. Resuscitation is carried out in accordance with AHA and NRP recommendations, which categorise it as initial and basic intervention, positive pressure ventilation, chest compressions, and medicine delivery. Poor birth weight, a short gestational period, a low Apgar score, and the need for prolonged resuscitation times all significantly influenced the level of resuscitation that newborns required. Additionally, the newborns that needed prolonged or advanced resuscitation exhibited signs of encephalopathy.[4]

Normal fetal circulation

There are many adaptive mechanisms a baby achieves physiologically after birth, one among it is the circulatory changes, delay or abnormal formations of these will cause pathological

condition and hypoxia, therefore fetal circulation is an important mechanism at birth. Oxygen is able to bind to foetal haemoglobin with a high affinity because it easily diffuses from the maternal circulation into the foetal circulation. The majority of the blood from the placenta that returns to the foetus through the umbilical vein flows through the ductus venosus. The blood's PO2 is between 40 and 50 mm Hg as it travels to the right atrium before it unites with blood from the inferior vena cava that has less oxygen in it. It's important to notice that the foramen ovale directs blood from the umbilical vein, which has a higher oxygen content, to the left heart. When the blood leaves the left ventricle of the heart, it passes through the aorta and into the coronary and carotid arteries. As a result, the foetus sends more oxygenated blood to the heart and brain, two parts of the body that need it the most. The superior vena cava's less oxygenated blood is pumped by the right ventricle into the pulmonary trunk. Major blood passes through the ductus arteriosus and the aorta as it leaves the lungs. Through the umbilical arteries, this blood mixture with a PO2 of 15 to 25 mm Hg reaches the placenta.[5,6]

The delivery of sufficient oxygen to meet the needs of the tissues is ensured by additional factors that are specific to the foetus. In comparison to adults and children, the levels of haemoglobin in a foetus are significantly higher. Because of its strong attraction to oxygen, foetal haemoglobin causes the oxygen–Hemoglobin dissociation curve to slope in the opposite direction, to the left. Because of this, oxygen can more easily be transferred from the mother to the foetus despite there being a low concentration gradient. These parameters result in an increase in the amount of oxygen that can be carried by the blood of the foetus. When compared to an adult, the rate of tissue perfusion in a foetus is significantly faster. As a result, a relatively low oxygen saturation can be remedied by increasing the delivery of blood. In addition, the foetus uses less energy than a newborn does, to maintain temperature regulation and respiratory effort.

Circulatory changes during labor and neonatal transition

The transition from life in the uterus to life outside the uterus causes significant changes in circulation. These transitions tend to occur in large numbers simultaneously. In the first few hours after birth, crying causes the lungs to rapidly expand, which lowers the pulmonary vascular resistance. The amount of blood passing through the lungs significantly rises. Right-to-left shunting at the ductus arteriosus starts to wane and then reverse as the pressure in the pulmonary artery drops below that of the systemic circulatory system. PaO2 increases encourage ductal closure. When this happens, the left atrium receives more blood than it did when the person was still a foetus because of the pulmonary venous system. The foramen ovale will become functionally closed when the left atrium's pressure exceeds that of the right atrium. The ductal shunt can be reversed thanks to raised systemic blood pressure brought on by an increase in systemic vascular resistance. A pattern in the adult circulatory system is found.

Causes of perinatal asphyxia

Before, during, or after delivery, there is a possibility of impaired gas exchange. The abnormal circulatory transition that results from this condition can happen at any time during labour and delivery. It is also possible for an infant to develop asphyxia in the neonatal period if the newborn is unable to maintain its own gas exchange in the absence of the placenta..[5]

The most common cause of asphyxia is an interruption of placental blood flow, which can happen both during pregnancy and labour and delivery. Blood flow interruptions can occur for a variety of reasons. Examples of maternal diseases that might alter the placental vasculature and lessen blood flow include diabetes, hypertension, and preeclampsia. Maternal hypotension may have an effect on the fetus's blood flow (eg; drug like Inj.MGSO4 , maternal APH).

Umbilical cord can get compressed from the outside, as seen in the cases of a cord around neck or cord prolapse. Asphyxia could also be caused by aspects that are unique to the newborns. For instance, abnormalities of the congenital airways may cause inadequate gas exchanges in lungs after the placental circulation has ceased. Neonatal patients with neurological abnormalities might not have the right amount of respiratory drive for effective ventilation. This may be due to a condition that is inherent to the neonate (such as an anomaly in the central nervous system or an injury to the spinal cord), or it may be due to the extrinsic effects of medications given to the mother.[7]

Maternal causes of Perinatal Asphyxia

- Diabetes mellitus
- Hypertension
- Preeclampsia
- Hypotension
- Uterine rupture
- Severe anaemia
- Infection

Placental/Umbilical cord factors

- Placental abruption
- Fetomaternal hemorrhage
- Infection/inflammation
- Velamentous cord insertion

Neonatal factors

- Airway anomalies
- Neurologic disorders
- Severe cardiopulmonary disease
- Severe circulatory compromise (blood loss)
- Infection
- Maternal Medication effect

Adaptive mechanisms after asphyxia

Some critical adaptations are induced in the developing baby when the blood flow via the placenta is disrupted, and these processes can either be circulatory or non-circulatory in origin. The shift of cardiac output and blood flow to important organs are two changes to the cardiovascular system. Non-circulatory reactions protect cell viability. End-organ damage is more likely when these adaptations are unable to keep up when the placenta's blood supply is significantly compromised.

Circulatory Changes After Asphyxia

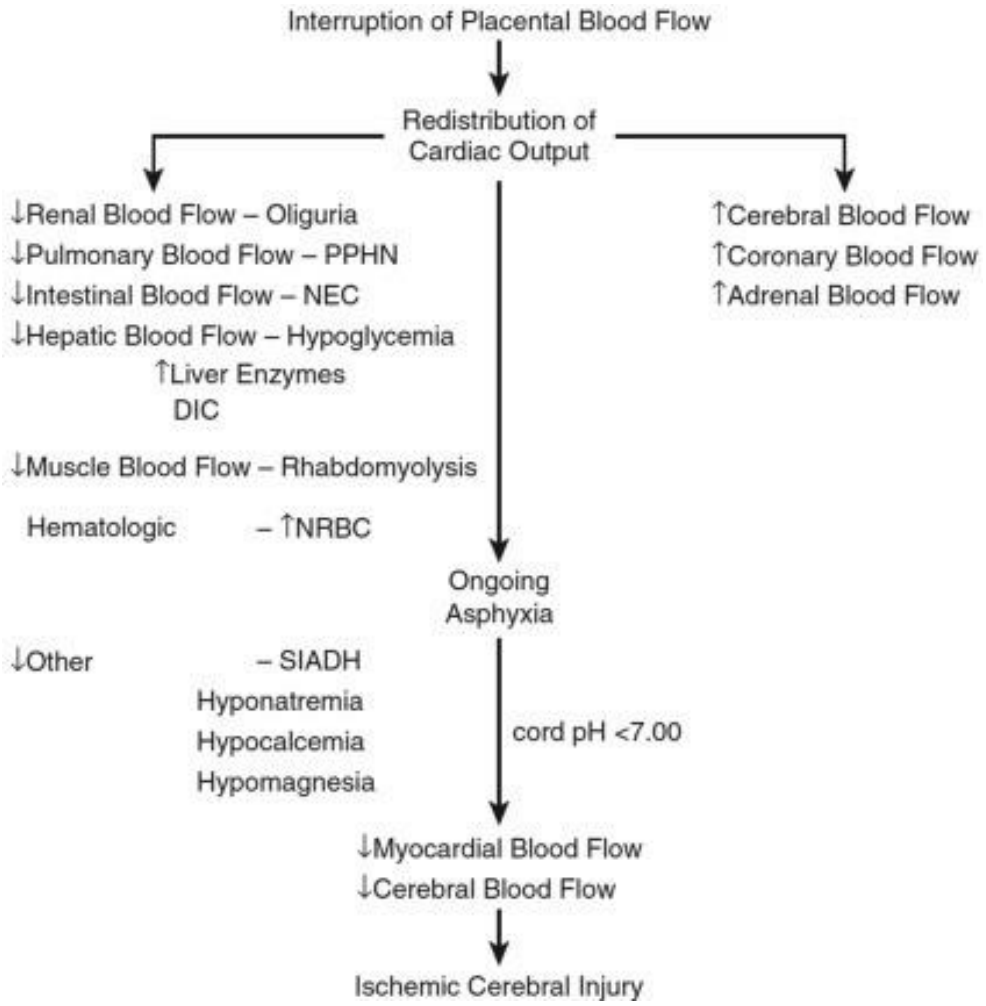
There are several different reasons that contribute to diving reflex. Chemoreceptors in the carotid artery detects hypoxemia, triggering the release of catecholamines. This rise in catecholamines triggers peripheral and a centralised blood flow as a reaction. Due to the pulmonary vasculature's constriction brought on by hypoxia, the left atrial pressure, and pulmonary blood flow are all decreased.[8,9]

In an effort to supply the left heart with even more oxygenated blood, blood is shunted from right to left through foramen ovale more frequently . Also, the cerebral circulation's adaptive mechanisms contribute to the enhancement of this process. As a result, when hypoxemia is present, cerebral vascular resistance will decrease.

The maintenance of blood flow to "critical" organs requires a reduction in blood flow to organs that are considered "noncritical." When the overall blood pressure in the body drops to the threshold level , compensatory mechanisms stop working. Brain damage happens as a result of insufficient oxygen being delivered to the brain due to increased dem

Fig 1: Adaptive mechanisms and systemic consequences of interruption of placental blood flow

and.

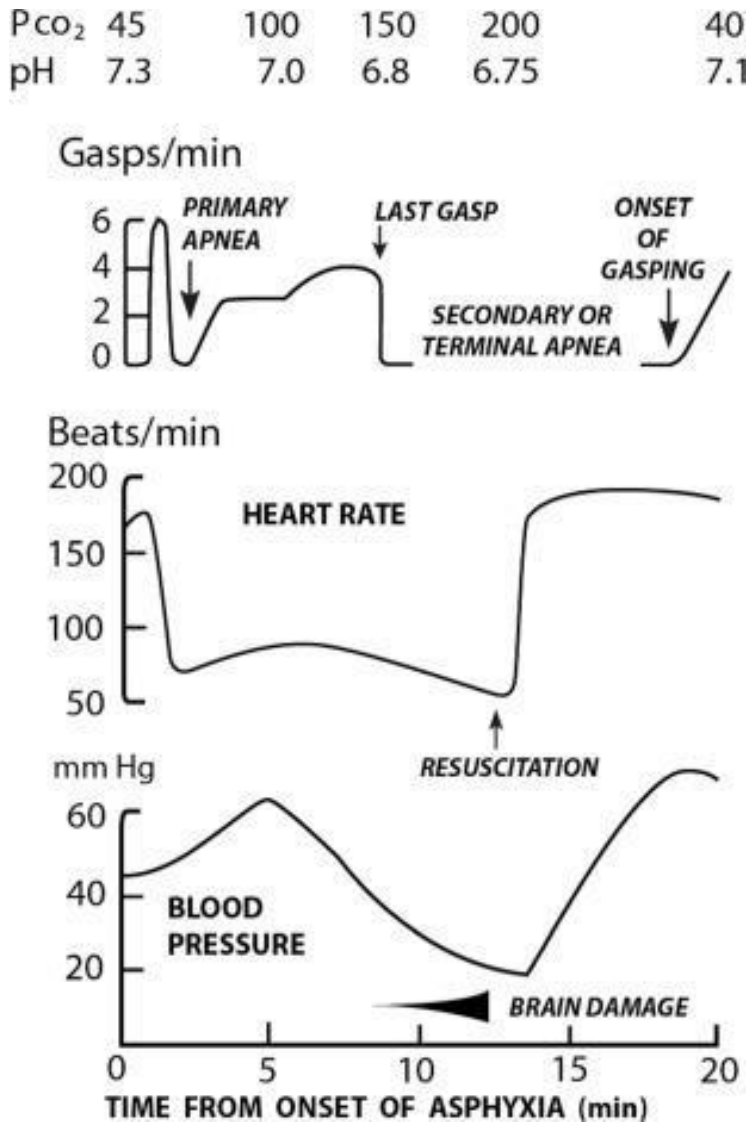


Even though the diving reflex is the best way to protect vital organ function, not all newborns ensure effectiveness these protective adaptation mechanisms.[10,11]

There was no multiorgan dysfunction in the 14 cases of hypoxic-ischemic encephalopathy (HIE) that Phelan and colleagues[11] documented. Cerebral palsy was found to be present in each of these neonates. The asphyxia-causing mechanisms in these circumstances, according to the theory, did not provide the foetal blood flow enough time to become centralised. According to research on both humans and animals, intermittent hypoxia lasting less than an hour is not known to cause cerebeal damage, but "complete" asphyxia will cause injury soon.[12,13]

Over a ten-years period, Shah and colleagues studied newborns with HIE. They discovered that all the children with severe post-asphyxial HIE had MOD. The result for these infants and MOD did not, however, correlate. There was no association between any particular organ involvement or combinations of organ involvements and long-term results.[10]

Mechanisms by heart in asphyxia



Non-circulatory Responses to Asphyxia

The viability of important organs is preserved both during and after hypoxia attributable to a number of physiological mechanisms. In comparison to term infants or adults, the brain metabolic rate is lower in foetuses, resulting in a more favourable balance between the supply and demand for energy.[14]

Impaired exchange of gases:

The characteristic of prenatal hypoxia is diminished oxygen and carbon dioxide gas exchange across the placenta. Simple diffusion is used by both gases to descend a partial pressure gradient. Acidosis is caused by a decrease in gas exchange[15-17].

The foetus, as previously mentioned, may thrive at relatively low oxygen pressures. Spiral arteries of the placenta get oxygenated blood from the maternal uterine artery. This blood mixes with deoxygenated blood when it reaches the relatively large intervillous area and comes into contact with the chorionic villi, which hold the foetal veins. Simple diffusion is a passive, non-energy-dependent method of oxygen delivery. In order to meet their energy needs, cells turn to anaerobic respiration when foetal oxygen demand exceeds placental oxygen delivery. Through this mechanism, lactic acid builds up and pH drops.

Major factors affecting placental oxygen transfer

Factor	Components
Placental membrane diffusing capacity	Surface area, thickness, oxygen solubility, diffusivity of tissues
Maternal arterial PO_2	Inspired PO_2, alveolar ventilation, mixed venous PO_2, pulmonary blood flow, pulmonary diffusing capacity
Fetal arterial PO_2	Maternal arterial PO_2, maternal placental Hb flow, placental diffusing capacity, umbilical venous PO_2, fetal O_2 consumption, fetal peripheral blood flow
Maternal and fetal Hb-O_2 affinities (P_{50})	pH, temperature, PCO_2, 2,3-diphosphoglycerate concentration, CO concentration
Maternal placental blood flow	Arterial pressure, placental resistance to blood flow, venous pressure
Fetal placental blood flow	Umbilical artery blood pressure, umbilical venous blood pressure, placental resistance to blood flow
Spatial relationship between maternal and fetal blood flow	Vascular architecture
Amount of CO_2 exchange	—

Abbreviations: CO-carbon monoxide; Hb -hemoglobin.

The foetus releases carbon dioxide, which is then delivered in the blood in three different ways: as dissolved gas, bicarbonate in red blood cells, and carbamate in haemoglobin. Dissolved CO2 gas is the main factor in placental transfer even though it contributes less to blood CO2 levels than bicarbonate and carbamate.[18]

It seems that CO2 diffuses around 20 times faster than oxygen. Blood flow is important because robust uteroplacental and fetoplacental circulations are necessary for carbon dioxide transfer.[18]

Before being discharged by the mother's lungs, the CO2 content changes from a higher foetal to a lower maternal level. This causes the maternal pH to be slightly (by around 0.1 units) higher than the foetal pH.

The Bohr and Haldane effects, two main phenomena, facilitate gas flow through the placenta. The accelerated oxygen transfer that is controlled by pH and PCO2 is known as the "Bohr effect." Maternal blood's oxygen-hemoglobin dissociation curve changes to the right as it takes in CO2 and becomes more acidic. As a result, oxygen is easier to unload and its affinity is reduced. The foetal circulation also experiences a loss of CO2 and increases in alkalinity, which causes the curve to move to the left and encourages oxygen intake.

The Haldane effect is a complimentary mechanism by which oxygen affects hemoglobin's ability to transport CO2. The increase in CO2 unloading on the foetal side is caused by oxygen binding to hemoglobin. As a result, the placenta has more foetal CO2 that can be transferred to the mother's bloodstream. Similar to this, when haemoglobin is deoxygenated, more CO2 can bind, assisting maternal circulation in the removal of CO2.

As a result, changes in foetal pH caused by an accumulation of carbon dioxide can happen and disappear fast. Noncarbonic acids, on the other hand, only gradually permeate the placenta and enter the maternal blood. Lactic acid, the main non-carbonic acid, builds up more gradually than carbonic acid as a result of anaerobic glycolysis and oxygen deprivation. A longer prolonged acidemia is the result of this process, and the degree of it may depend on the severity and length of the hypoxic-ischemic insult.[19]

Asphyxia is still not clearly defined in terms of umbilical arterial pH or the degree of acidosis. A pH of less than 7.20 in the cord-umbilical arterial fluid was previously used to define asphyxia. A pH of less than 7.00 in the umbilical arterial blood indicates severe foetal acidemia, which increases the likelihood of negative neurologic effects. [20,21] Even with this level of academia, the chance of further brain damage is still slim.

A benign neurologic outcome is seen in 80% to 90% of newborns with severe foetal acidemia who are admitted to critical care (typically because of respiratory problems), and only a small percentage develop encephalopathy.[22–24]

In a research, 8 of 47 babies with severe foetal acidemia who were hospitalised to the critical care unit experienced HIE, which included seizures.[22] This study found that the likelihood that newborns with seizures would require cardiopulmonary resuscitation in the delivery room was 234 times higher than the likelihood of infants without seizures.[22] As a result, whereas high foetal acidemia is unmistakably a sign of stress, this does not necessarily suggest that the foetus cannot maintain cerebral perfusion. However, a significant intrapartum insult is more likely to occur when substantial acidemia is seen alongside a bradycardic baby who requires emergency delivery room resuscitation. In this case, cerebral perfusion as well as the supply of oxygen to the brain were both compromised. Due in part to the foetus' ability to adapt to disruptions in placental blood flow in order to maintain cerebral perfusion and oxygen delivery, the brain exhibits an incredible resistance to asphyxia, even in the case of severe cases.

Neuronal cell death after asphyxia

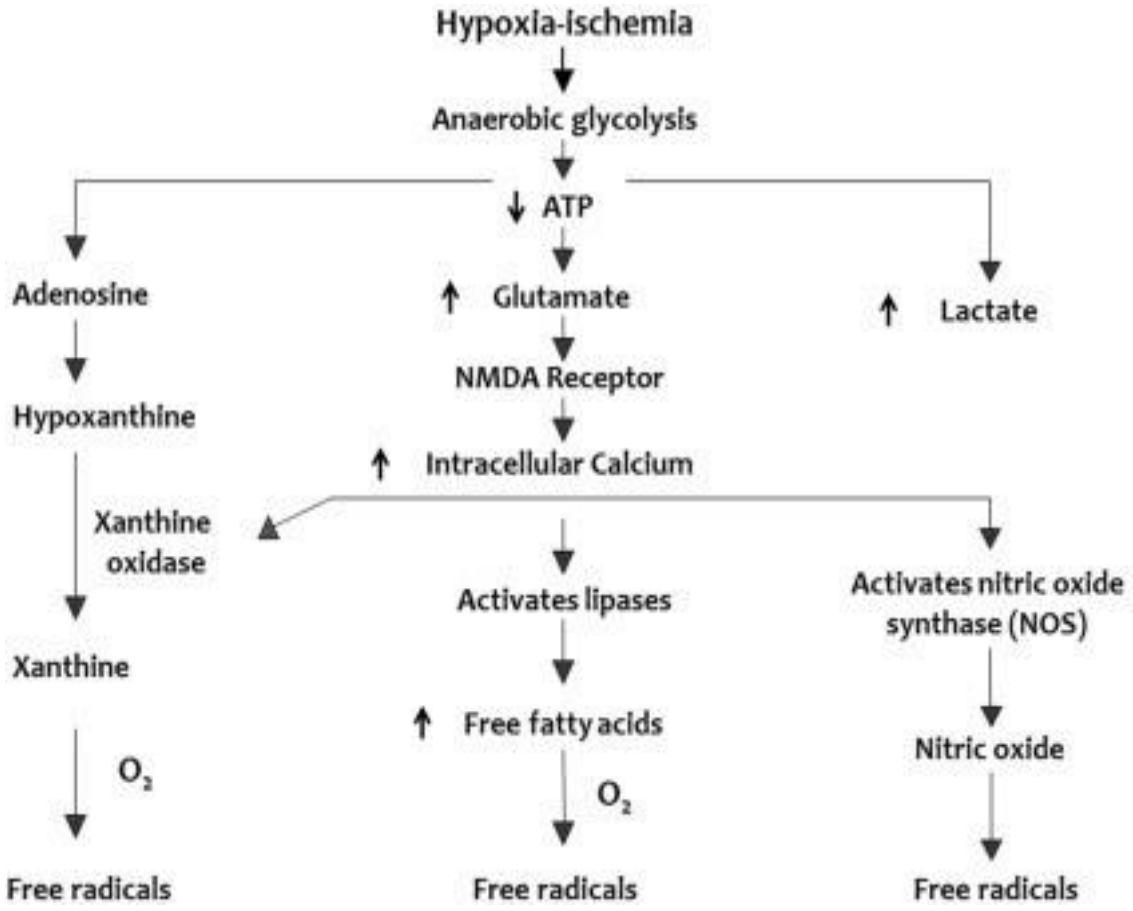
When few body mechanisms fail and cerebral perfusion is insufficient to meet demand, a number of metabolic responses begin. If not stopped, these intricate, interconnected processes eventually lead to cell death. The cellular biology of asphyxia-related hypoxic-ischemic brain injury is the main topic of this section.

Anaerobic glycolysis replaces aerobic glycolysis and high-energy phosphate molecules decline in the asphyxiated foetus or neonate (ie; adenosine triphosphate and phosphor creatinine). Na + /K + adenosine triphosphatase and Na + /Ca 2+ exchanger membrane ion pumps malfunction, causing lactic acid to build up. Failure of the membrane pump causes an influx of salt and water into the cells, which causes cell swelling. Additionally, calcium enters cells, starting the process by which glutamate and other excitatory amino acids are released into the extracellular environment. An excitotoxic cycle is aided by the additional calcium influx that results from this overexcitation.[25] Free radical generation, nitric oxide production, and lipid peroxidation of cell membranes are further effects.

Classically, necrosis or apoptosis are regarded as the final stages of cell death (programmed cell death). Cell enlargement, organelle disintegration, and phospholipid membrane integrity loss with cell lysis are characteristics of necrosis. It is an indication of the primary hypoxic-ischemic insult, which causes a quick and severe collapse of cellular function.[26] Following resuscitation, cerebral perfusion, oxygenation, and some energy sources are partially recovered. A secondary energy failure, or further progressive drop in high-energy phosphates, occurs 24 to 48 hours later.[27]

Reperfusion damage develops as a result of prolonged responses to the first insult during subsequent energy loss. Reactive oxygen species and free radical production, as well as cell death by apoptosis, are all characteristics of this damage. 28 Adenosine triphosphate is actively employed to break down cells into useable parts when apoptotic pathways are activated.[26] Chromatin condenses, cells contract, and nuclei have pyknotic morphology. Caspase-dependent or caspase-independent pathways for gene transcription can cause apoptosis.[25] Caspase-3 is effective caspase in the developing brain, and its activation during hypoxia-

ischemia is directly connected with the severity of the damage. Apoptosis has emerged as a tempting target for prospective HIE treatments due to its delayed nature.[29] Hybrid neuronal death processes have attracted attention recently.



perinatal asphyxia's onset and duration

Parents, neonatologists, and obstetricians frequently place a great deal of emphasis on the precise moment an asphyxia episode took place. This may be clear-cut in situations where there have been significant sentinel events, such as uterine rupture, placental abruption, cord prolapse, or trauma. This, however, is elusive in some circumstances. Both acute and subacute asphyxia insults fall within this classification.

A "megacode," in which a complete resuscitation takes place, is a well-known illustration of an intense asphyxia injury.[31] The baby had poor Apgar scores and severe acidosis when it was born, and there may have been a sudden change in the foetal heart rate that was noticed. Encephalopathy frequently coexists with malfunction of the kidneys and other end organs.

A portion of new-borns who have suffered from asphyxiation might not have had a serious case of circulatory collapse. These incidents suggest that the injury was subacute, allowing the foetus to "self-resuscitate" inside the mother. A lot of the time, labour is straightforward, and the new born is delivered without any severe complications. As a result, severe acidaemia is concealed, however encephalopathy may be present. Within 12 to 24 hours, some of these infants may not be observed at first but develop encephalopathy and convulsions..[32]

A recent study of term infants receiving hypothermia treatment described a substantially different appearance. Seven children with subacute insults exhibited more severe encephalopathy at delivery and were less likely to require intensive resuscitation than the 26 newborns with acute injuries (eg, uterine rupture).[33] Both presentations frequently involve systemic organ damage, especially renal impairment, as well as MRI indications of a brain injury. The injury's timing is frequently problematic in these situations. Along with typical MRI abnormalities, inconspicuous signals from the maternal history, such as decreased foetal movement, may be helpful. Injury on an MRI may change during the course of the reperfusion period, so this should be considered when interpreting the image. [34] For instance, until day 4 or 5, diffusion and metabolic abnormalities became worse before starting to normalise.[35]

The duration of hypoxia can be inferred from specific injury patterns. Diffuse neuronal damage frequently results from the harsh and longest-lasting insults. From mild to severe chronic insults, cortical and deep nuclear (basal ganglia and thalamic) neuronal damage is

frequently the end result. Deep nuclear-brain stem injury is primarily brought about by abrupt, severe hypoxia-ischemia.

Oxidative Stress

Under pathologic conditions, a rise in the production of free radicals as a result of oxidative metabolism causes alterations in the cellular environment that are referred to as "oxidative stress.". Excitotoxicity and oxidative stress are intricately related in the brain damaged by hypoxia-ischemia. Cytochrome oxidase turns more than 80% of the available oxygen in cells with healthy mitochondria into energy equivalents (adenosine triphosphate [ATP]). The remaining portion is transformed into superoxide anions, which are then reduced to water under physiological conditions by enzymatic and nonenzymatic antioxidant processes.

A build-up of superoxide is an unavoidable side effect of mitochondrial malfunction, and any procedure that exhausts antioxidant defences will cause superoxide to automatically transform into even more dangerous species, including the hydroxyl radical.[36] Understanding oxidative stress requires an understanding of ischemia-reperfusion injury, or the evolution of tissue damage with reoxygenation after ischemia. Excitotoxic brain mechanisms can amplify these actions even though this mechanism is not exclusive to the brain. Excitotoxicity causes energy loss, mitochondrial dysfunction, and cytosolic calcium accumulation in addition to the formation of free radicals such superoxide, nitric oxide derivatives, and the particularly reactive hydroxyl radical.

During reoxygenation, reactive oxygen species accumulate and mitochondrial oxidative phosphorylation becomes overworked. Numerous biological components, including lipids, DNA, and proteins, are directly damaged by free radicals. As innate antioxidant defences are depleted, free radicals can also start proapoptotic pathways. The brain is especially susceptible to the harm caused by free radicals and lipid peroxidation, and this sensitivity is increased in the growing brain. The presence of higher free-iron concentrations compared to adult brains, high levels of lipid peroxidation (particularly in response to hypoxic stress), a high proportion of polyunsaturated fatty acids, and the immaturity of antioxidant defence enzymes are all contributing factors.

Infants who have died from asphyxiation have increased levels of free iron in their plasma and brain fluid, which catalyses the production of several reactive oxygen species. The detrimental effects of too much iron and insufficient enzymatic antioxidant defences in the developing brain are closely related. Alpha-phenyl-n-tert-butyl-nitrone [PBN], a spin-trap agent that converts free radicals into stable adducts, is a free-radical scavenger that can protect neurons against hydrogen peroxide injury both in vitro and in vivo. The fact that these medications also protect neurons from NMDA-induced toxicity serves as more evidence of the pathophysiologic relationship between excitotoxicity and oxidative stress.

Nitric oxide metabolism contributes to the relationship between excitotoxicity and oxidative damage in the hypoxic-ischemic-damaged brain. A rise in intracellular calcium triggers the constitutive production of nitric oxide in the endothelium, astrocytes, and neurons. The creation of more nitric oxide during hypoxic-ischemic conditions can have a variety of positive and negative impacts. Nitric oxide (NO) controls the tone of the blood vessels, affects how the body reacts to injury-related inflammation, and directly affects how well NMDA receptors work.

A study found that striatal neurons that express nNOS are disproportionately resistant to the effects of hypoxia-ischemia, acting as a source for NMDA receptor-mediated regulation of nitric oxide generation. Pharmacological nNOS suppression and nNOS gene disruption reduce the damages caused by neonatal hypoxia-ischemia. Distinct nitric oxide synthase isoforms may provide a different treatment approach for protection while also being able to cause damage through inflammation (iNOS). Early endothelial NO is protective because it maintains blood flow, but early neuronal NO and late inducible NO are neurotoxic because they promote cell death. Following brain injury, multiple cell types are encouraged to produce brain iNOS, which enhances excitotoxicity through altering binding to NMDA receptors.[37]

It has been proven that specific suppression of nNOS or iNOS provides neuroprotection. NMDA receptor expression and nNOS expression are correlated with areas of neurotoxicity both in vivo and in vitro.

There aren't many research on cerebral NO production in asphyxiated human infants. 24 to 72 hours after hypoxia, CSF NO levels rise along with the severity of HIE, and the spinal cord's NO and nitrotyrosine levels also rise.[38–40]

However, a significant European experiment found no difference in neurodevelopmental or other health outcomes at 2 years of age with low-dose (5 ppm) iNOS administered early (24 hours after birth) for a median of 20 days. Initial outcomes at 2 years of age in preterm infants treated with inhaled NO for bronchopulmonary dysplasia prevention demonstrated reductions in brain injury as determined by ultrasonography and improvements in neurodevelopmental outcomes.

There are various different antioxidant methods that have been researched that either improve antioxidant defences or limit free-radical generation. Melatonin, which exhibits effectiveness in both preterm and term damage, appears to be the most promising of the antioxidant medications. Melatonin targets multiple processes along the damage cascade, including oxidative stress, inflammation, apoptosis, mitochondrial failure, and nuclear impact. The indoleamine melatonin, which is produced in greater amounts in adults, directly scavenges ROS and NO. In experimental HI and focal cerebral ischemia injury, it has been demonstrated to provide sustained neuroprotection. Melatonin treatment in human neonates was similarly found to reduce levels of pro-inflammatory cytokines.[41]

Within the first six hours of life, melatonin administration to babies who had been asphyxiated by humans caused a decrease in the levels of oxidative compounds in their serum, including MDA and nitrite/nitrate 42. In specifically, they gave ten asphyxiated babies an oral dose of 80 mg of melatonin (eight doses of 10 mg, each spaced by 2 hours). After a blood sample was taken, one blood sample was taken before melatonin was given, and two more blood samples—at 12 and 24 hours—were taken after indolamine was given. It was discovered that newborns with hypoxia had serum concentrations of MDA and nitrite/nitrate that were considerably greater than those of infants without asphyxia before therapy. At both 12 and 24 hours, the MDA and nitrite/nitrate levels in the asphyxiated babies who had received melatonin significantly decreased. Researchers verified that melatonin has antioxidant characteristics and can enhance mitochondrial electron transport.[42]

In research on both animals and people, allopurinol has conflicting effects but also shows potential. Superoxide and H2O2 produced by xanthine oxidase react with NO to produce harmful reactive nitrogen species (RNS). Inhibiting xanthine oxidase and scavenging hydroxyl

radicals are how allopurinol reduces the generation of free radicals. Short-term advantages have also been shown in neonatal infants who underwent cardiac surgery for hypoplastic left heart syndrome.

Neuroinflammation

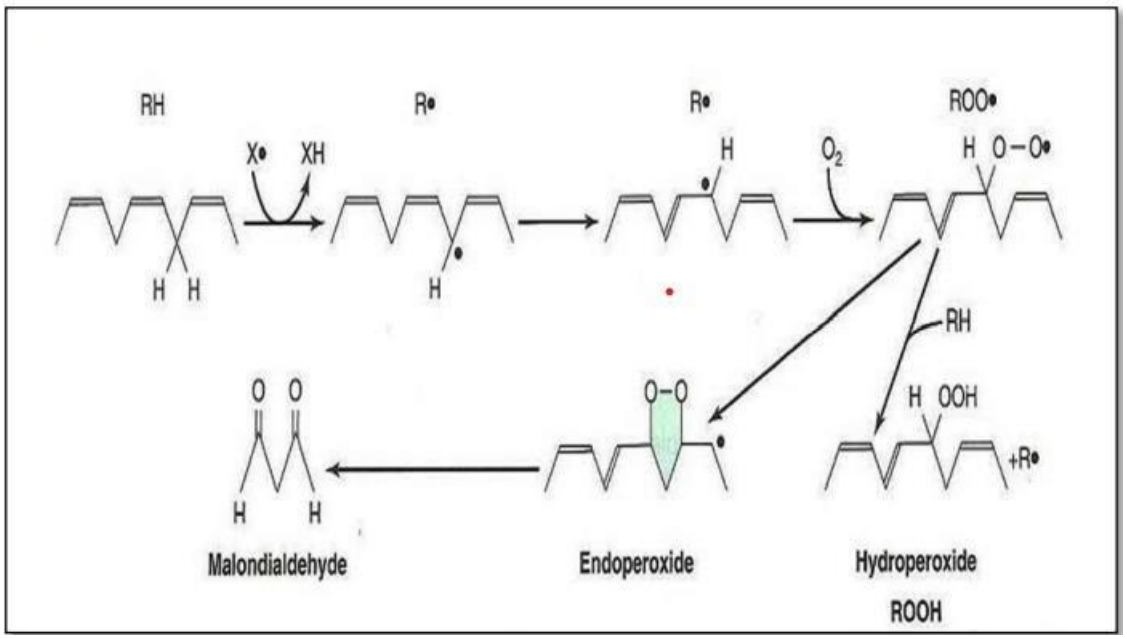
Following neonatal hypoxia-ischemia, inflammation has emerged as a critical regulator of the damage response. The excitotoxic cascade is aided by mediators such as cytokines and chemokines, which work with free radicals and NO in an immunological response to prenatal brain injury. The cytokines Interleukin-1b, Tumour Necrosis Factor-alpha, Interleukin-6, and Membrane Cofactor Protein-1 have been closely associated with their involvement in mediating inflammation in the developing brain. There are numerous potential sources of plasma cytokines after an asphyxia episode, including damaged endothelium and severely wounded organs, like the brain via a ruptured blood-brain barrier. Asphyxiated term new-borns' CSF and plasma levels of several cytokines have been measured, and The findings suggest that drastically elevated cytokine levels may be related to the injured brain. The concept that at-risk newborns have an inherited susceptibility to cerebral palsy is supported by a more recent study that linked IL-6 to the disorder.[43,44]

There is still some controversy despite the established connection between maternal sickness and infant brain injury. In a case-control study from the Kaiser Permanente Medical Care Program, the association between clinical chorioamnionitis and risk of cerebral palsy was examined using a file review of kids with mild to moderate spastic or dyskinetic cerebral palsy. The study found a four-fold overall increase in the incidence of cerebral palsy in term babies due to chorioamnionitis or placental infection.[45]

According on the source of the infection, there is a clear difference in outcomes for term children with neonatal encephalopathy and signs of chorioamnionitis or sepsis in the new born (infant infection). According to MRI findings, neonates exposed to chorioamnionitis had a lower risk of brain damage and adverse outcomes than babies with sepsis who had a higher likelihood of suffering mostly watershed injuries.[46] In a different cohort of term neonates with HIE, reduced placental maturation indicated a higher risk of white-matter watershed injury, while persistent villitis was connected to impairment of the basal ganglia and thalamus irrespective of white-matter injury. It is necessary to pay more attention to placental pathology when evaluating a new born who has NE and likely has HIE. The greatest marker of lipid peroxidation and hence of oxidative stress is MDA.[47] An essential part of the aetiology of many diseases is demonstrated by oxidative stress. [48]

MALONDIALDEHYDE AS A MARKER FOR LIPID PEROXIDATION

An outcome of oxidative stress is the end-product malondialdehyde (MDA). It is one of the several compounds created when the phospholipids in the cell membrane degrade. Arachidonic acid (AA), which is generated as a result of phospholipase-A2 activity, is then attacked by ROS (hydroxyl radical OH•) from mitochondria through a non-enzymatic process, resulting in the formation of lipid endoperoxide. This lipid endoperoxide spontaneously bursts, forming MDA inside the cell. MDA is eventually released into the circulation after first entering extracellular space. [49]



USING MALONDIALDEHYDE AS A BIOMARKER

Monitoring MDA levels in many biological systems can be used as a vital in-vitro and in-vivo lipid peroxidation biomarker for a number of disorders. Due to its function in the endogenous formation of DNA adducts during intracellular oxidative stress, MDA is a major biomarker of endogenous DNA damage.[50]

Measuring the MDA in blood plasma or tissue homogenates is a useful method for predicting the levels of oxidative stress. Thiobarbituric acid reactive substance (TBARS), a marker of lipid peroxidation, includes MDA as one of its types. MDA levels are assessed in a range of samples, including serum, plasma, and tissues, using a number of techniques. The thiobarbituric acid (TBA) assay is the procedure that is most frequently used to determine MDA.

TBA assay is a non-specific test for MDA since it reacts with other aldehydes that could be present in biological materials. These chemicals also generate coloured species that interfere with the MDA assay. For the purpose of isolating and quantifying the MDA-TBA adduct, novel analytical methods, such as gas chromatography mass spectrometry (GC-MS) and high-

performance liquid chromatography (HPLC), have been developed. Higher MDA levels were found to be caused by hydrogen peroxide (H2O2) and tert-butyl hydroperoxide in hepatoma cell (HepG2) culture (t-BOOH).[51]

Derivatization of MDA using 2, 4-dinitrophenylhydrazine (DNPH) has also been described for detecting MDA levels in human urine.[52]

In 2008, Ashok Kumar et al. examined 50 term infants who had perinatal asphyxia and eight infants who had not. Infants under the age of one who had sepsis, severe congenital defects, or haemolytic illness were not included. In this study, new-borns who had experienced perinatal asphyxia had significantly higher levels of plasma and cerebrospinal fluid malondialdehyde, as well as plasma glutathione peroxidase, catalase, and superoxide dismutase. These levels also showed a progressive increase with greater severity of hypoxic ischemic encephalopathy. Neonates who died from hypoxia ischemic encephalopathy showed higher levels of malondialdehyde and plasma catalase compared to new-borns who survived the disease. Blood levels of superoxide dismutase and glutathione peroxidase, however, were unaltered. The findings of this study suggest that prenatal hypoxia causes antioxidant enzymes to function more effectively, but that oxidative stress is nonetheless more severe in these new-borns, as evidenced by greater levels of malondialdehyde in both plasma and cerebral fluid. Consequently, it can be said that oxygen free radicals are very important in the pathophysiology of neonatal hypoxia.[53]

SARAT AND SARNAT STAGES OF HYPOXIC ISCHEMIC ENCEPHALOPATHY[55]

Stage	Stage 1 (Mild)	Stage 2 (Moderate)	Stage 3 (Severe)
Level of consciousness	Hyperalert; irritable	Lethargic or obtunded	Stuporous, comatose
Neuromuscular control:	Uninhibited, overreactive	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	Midposition, often unequal; poor light reflex
Respirations	Spontaneous	Spontaneous; occasional apnea	Periodic; apnea
Heart rate	Tachycardia	Bradycardia	Variable
Bronchial and salivary secretions	Sparse	Profuse	Variable

Staging continued on following page..

Gastrointestinal motility	Normal or decreased	Increased, diarrhea	Variable
Seizures	None	Common focal or multifocal (6-24 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; 1.0-1.5 Hz spike and wave	Later: totally isopotential
Duration of symptoms	<24 hours	2-14 days	Hours to weeks
Outcome	About 100% normal	80% normal; abnormal if symptoms more than 5-7 days	About 50% die; remainder with severe sequelae

MATERIALS AND METHODS

This study was carried out on 102 newborn babies at BLDE (DU's) Shri B M Patil Medical College, Hospital & Research Centre, Vijayapura between January 2020 and June 2022 who met inclusion criteria.

INCLUSION CRITERIA:

All newborns delivered did not require resuscitation as controls and who required resuscitation as cases at Shri BM Patil hospital irrespective of gestational age and mode of delivery.

EXCLUSION CRITERIA:

- Neonates with gross congenital anomalies.
- Newborns discharged against medical advice.
- Babies whose cord blood couldn't be collected

STATISTICAL ANALYSIS

- We categorized the babies based on the level of neonatal resuscitation method used correlating the maternal risk factors.
- Cord blood samples were taken at the time of birth

- Sample size calculation:
- With anticipated Mean± SD of MDA level in new born 4.38+/-0.28, the study would
- A sample size of 100 newborns with 95% level of confidence and a precision of 10%.

- Formula used
- n=z 2- S 2/d2
- Where Z= Z statistic at α level of significance
- d 2 = Absolute error
- P= Proportion rate
- q= 100-p
- Statistical Analysis:
- The data obtained was entered in a Microsoft Excel sheet, and statistical analysis will be
- performed using statistical package for the social sciences (Version 20)
- **Type of study**: **A prospective observational study.**

ESTIMATION OF MDA IN CORD BLOOD

PRINCIPLE: MDA is formed by the breakdown of polyunsaturated fatty acids (PUFA) serves as a convenient index to determine the extent of lipid peroxidation. It reacts with TBA to give a pink colour which is read at 535nm[56]

Sample: Serum-100µL

Chemicals required:

- Tri chloro acetic acid (TCA)-(CH3COOCl3)
- 2-Thiobarbituric acid (TBA)-($C_4H_4N_2O_2S$)
- Hydrochloric acid (HCl)
- Malondialdehyde (dimethyl acetal) – ($C_7H_{16}O_4$)

PROCEDURE :

⇨ The serum-100µL serum is diluted to 500µL with distilled water.

⇨ 1ml of TCA-TBA-HCl reagent added to the diluted sample.

⇨ The samples were placed in a boiling water bath for 15 minutes.

⇨ The reaction mixture is then cooled and centrifuged.

⇨ At 535nm, the supernatant was taken, and the optical density of the pink colour formed was read.

- ⇨ The plotting the obtained absorbance against the standard graph to get a concentration of malondialdehyde in the sample.
- ⇨ The optical density of the pink colour formed is directly proportional to the concentration of malondialdehyde in the given sample in micromole/L

PICTURE 1: PINK COLOUR SUPERNATANT

PICTURE 2: Photo-spectrometer for reading at 535nm

In our study we have selected 102 newborns randomly whose cord blood could be collected and delivered at BLDE medical college and correlated the maternal risk factors with the need for resuscitation , the newborns resuscitated were analysed with the levels of resuscitation required and categorised into mild moderate severe asphyxia ,based on sarnat staging . The cord blood collected was centrifuged and serum separated , and stored at -20degrees C, MDA was assessed and the correlations were done.

The Centres for Disease Control and Prevention define anemia in iron-supplemented pregnant women using a cut-off of 11g/dL in the first trimester and 10.5 g/dL in the second trimester . we have taken the hemoglobin values of less than 10 as significant in our study

Placenta previa describes a placenta that is implanted somewhere in the lower segment, either over or very near the internal cervical Os.

Placenta previa – the internal os is covered partially or completely by placenta

Low lying placenta- implantation in the lower uterine segment is such that the placental edge does not cover the internal Os but lies within 2cm perimeter

The classification will also depend on the cervical dilatation at the time of presentation, in our study we have considered the placenta previa that was identified in sonography and diagnosed by the obstetricians.

Pre-eclampsia is a pregnancy specific syndrome that can affect virtually every organ system, the following criteria is used for diagnosis of the condition

Condition	Criteria Required
Gestational hypertension	BP >140/90 mm Hg after 20 weeks in previously normotensive women
Preeclampsia: Hypertension plus	
Proteinuria	• ≥300 mg/24 h, or • Urine protein: creatinine ratio ≥0.3, or • Dipstick 1+ persistent[a]
	or
Thrombocytopenia	• Platelet count <100,000/μL
Renal insufficiency	• Creatinine level >1.1 mg/dL or doubling of baseline[b]
Liver involvement	• Serum transaminase levels[c] twice normal
Cerebral symptoms	• Headache, visual disturbances, convulsions
Pulmonary edema	—

RESULTS

Table 1: Comparison of basic demographic and Obstetric characteristics of mothers, of the Newborns with and without birth asphyxia (N=102)

Characteristics	Newborns with Birth Asphyxia	Newborns without Birth Asphyxia	Total Newborns	p-value
Sex of the baby				
Male	20 (32.8%)	41 (67.2%)	61 (100%)	0.234
Female	9 (21.9%)	32 (78.1%)	41 (100%)	
Gestational age				
37 – 40 weeks	25 (86.2%)	57 (78.1%)	82 (80.4%)	0.351
>40 weeks	4 (20.0%)	16 (80.0%)	20 (100%)	
Parity				
Primi	10 (24.4%)	31 (75.6%)	41 (100%)	0.458
Multi	19 (31.2%)	42 (68.8%)	61 (100%)	
Mean (SD) maternal age	**26.5 (3.7)**	**26.2 (3.8)**	**26.3 (3.7)**	**0.693**
20 – 24 years	10 (25.0%)	30 (75.0%)	40 (100%)	0.826
25 – 29 years	12 (30.8%)	27 (69.2%)	39 (100%)	
30 – 36 years	7 (30.4%)	16 (69.6%)	23 (100%)	

In our study 29 neonates were with birth asphyxia and 73 were without birth asphyxia , with 32% males in with birth asphyxia and 67% without asphyxia . Gestational age is between 37-40 weeks in both the groups , 20 newborns with >40 weeks gestational age , 4 with birth asphyxia and 16 were without birth asphyxia , Mean maternal age is 26.3

Table 2: Comparison of Pregnancy related complications among Newborns with and without birth asphyxia (N=102)

Pregnancy related Complications	Newborns with Birth Asphyxia(N=29)	Newborns without Birth Asphyxia(N=73)	Total Newborns	p-value
Anaemia	3 (42.9%)	4 (57.1%)	7 (100%)	0.381
Pre-eclampsia	6 (54.5%)	5 (45.5%)	11 (100%)	**0.042**
PROM	6 (46.2%)	7 (53.8%)	13 (100%)	0.129
Placenta Previa	7 (77.8%)	2 (22.2%)	9 (100%)	**0.001**
History of sub-fertility	1 (33.3%)	2 (66.7%)	3 (100%)	0.848
Prolonged II stage	9 (64.3%)	5 (35.7%)	14 (100%)	**0.001**
Gestational diabetes mellitus	0	4 (100%)	4 (100%)	0.198
Heart disease	0	0	0	NA
UTI	1 (50%)	1 (50%)	2 (100%)	0.495
Vaginitis*	2 (66.7%)	1 (33.3%)	3 (100%)	0.136

In our study there was significant correlation of maternal factors with birth asphyxia associated with pre-eclampsia, placenta previa and prolonged second stage of labour, there was also multifactorial causation found in our cohorts

***E.Coli in all 3**

Figure 1: Comparison of Pregnancy related complications among Newborns with and without birth asphyxia (N=102)

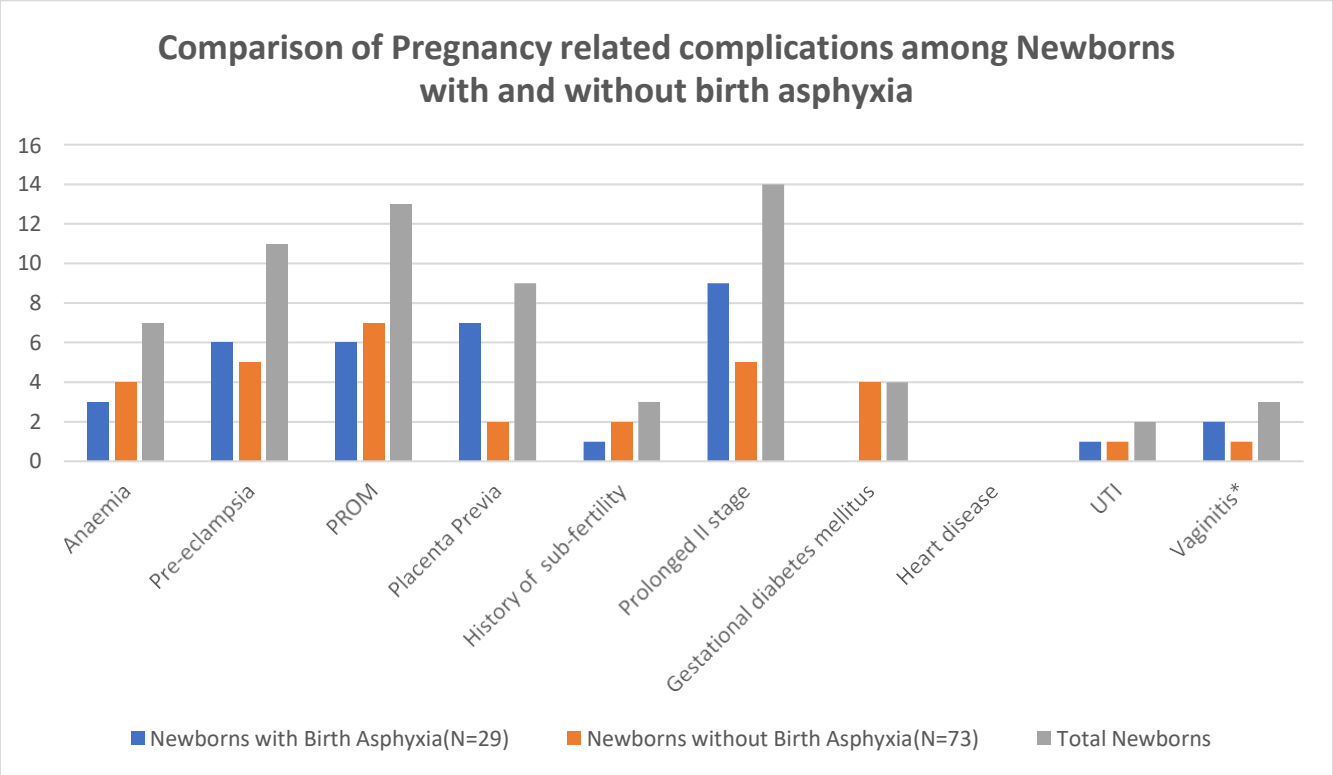


Table 3: Comparison of Delivery related factors among Newborns with and without birth asphyxia (N=102)

Delivery related factors	Newborn with Birth Asphyxia	Newborn without Birth Asphyxia	Total Newborn	p-value
Amniotic fluid				
Meconium stain	11 (57.9%)	8 (42.1%)	19 (100%)	**0.001**
Clear	17 (57.9%)	65 (79.3%)	82 (100%)	
Mode of delivery				
LSCS	16 (29.6%)	38 (70.4%)	54 (100%)	0.539
Vaginal	13 (28.9%)	35 (72.9%)	48 (100%)	
Birth weight				

1500 – 2500 gm	8 (80.0%)	2 (20.0%)	10 (100%)	**<0.001**
2500 – 3500 gm	21 (22.8%)	71 (77.2%)	92 (100%)	
Appropriate for gestational age				
LGA	1 (100%)	0	1 (100%)	**<0.001**
SGA	8 (80.0%)	2 (20.0%)	10 (100%)	
AGA	20 (22.0%)	71 (78.0%)	91 (100%)	

In our study the delivery related complications like mecomium stained amniotic fluid, low birth weight and SGA were significantly associated with birth asphyxia

Figure 2 : Comparison of mode of delivery among newborn with and without Asphyxia

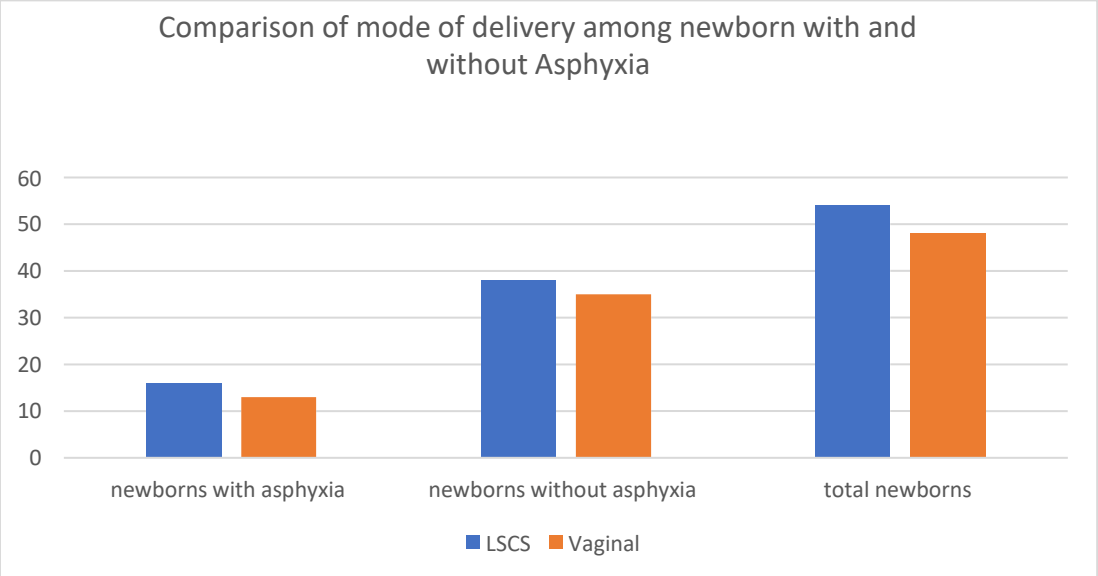


Figure 3: Comparison of birth weight among Newborns

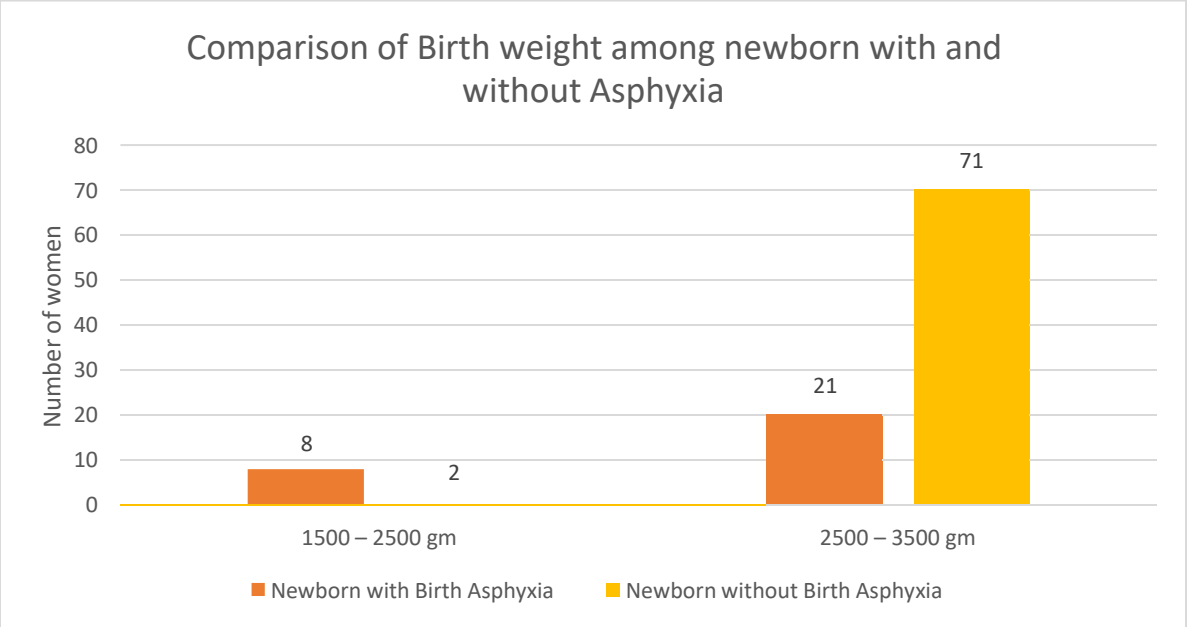


Figure 4 : Comparison of Appropriate for gestation among Newborns with and without Asphyxia

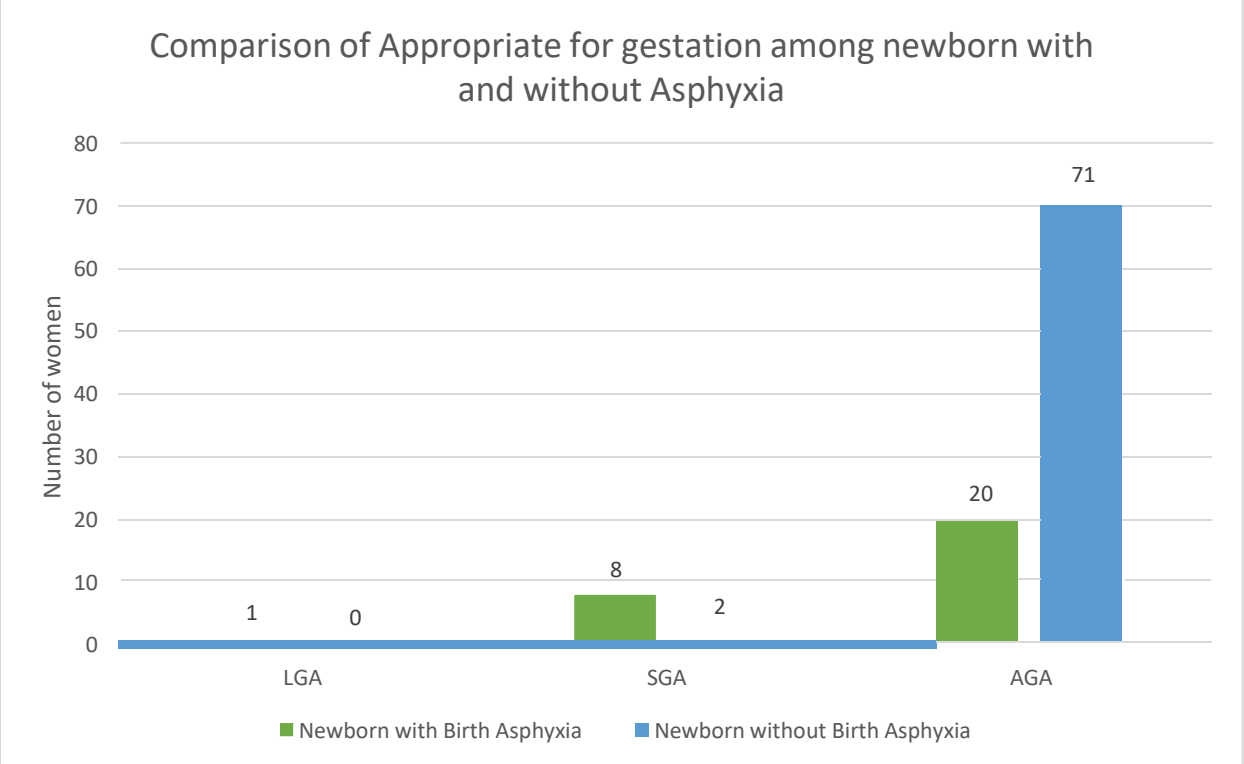


Table 4: Comparison of APGAR score among Newborns with and without birth asphyxia (N=102)

APGAR score	Newborns with Birth Asphyxia	Newborns with mild/No Birth Asphyxia	Total Newborns	p-value
At birth				
<=3	5 (17.2%)	0	5 (4.9%)	<0.001
4-6	24 (82.8%)	0	24 (23.5%)	
>=7	0	73 (100%)	73 (71.6%)	
At 5 mins				
<7	7 (100%)	0	7 (100%)	<0.001
≥ 7	22 (23.2%)	73 (76.8%)	95 (100%)	

In our study we considered APGAR score <3 as severe, 4-6 moderate and more than 7 as normal, newborns with asphyxia are with APGAR score <= 6 at 1 min. APGAR <=3 are seen in 5, 4-6 in 24 neonates and normal (>=7) in 73 neonates

Figure 5: Comparison of APGAR score among Newborns with and without birth asphyxia (N=102)

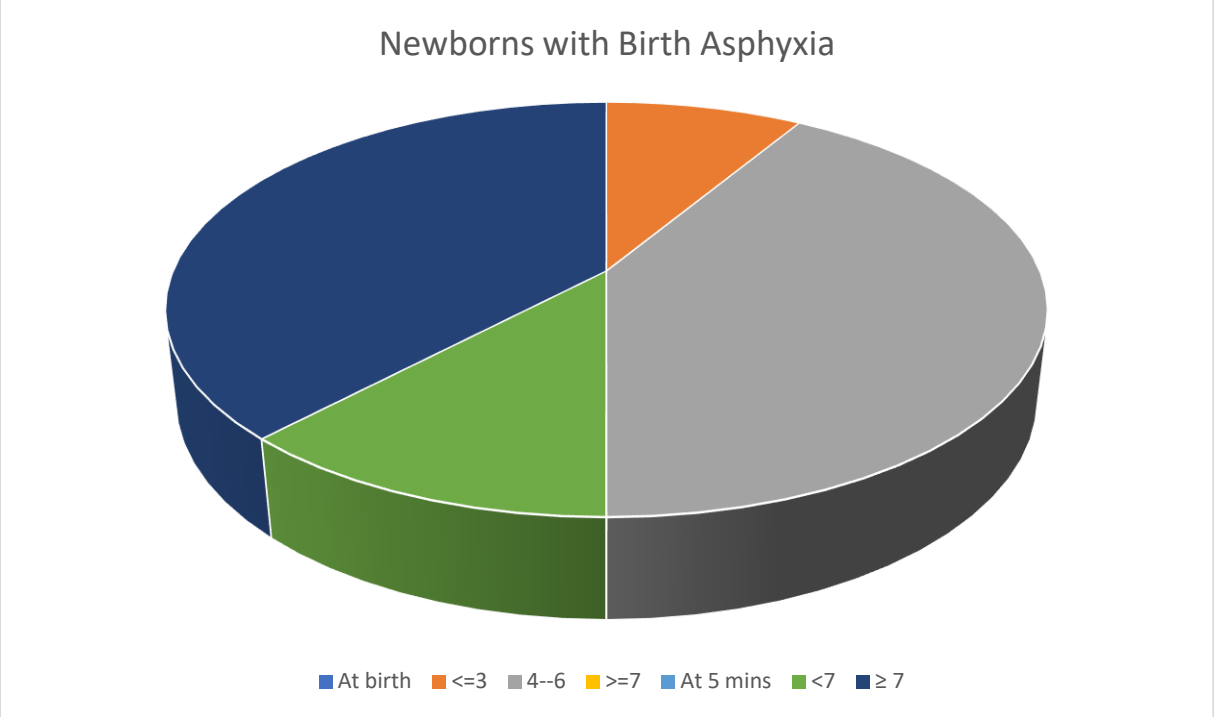


Table 5: Comparison of complication among Newborns with Sarnat staging (N=102)

Complications\Asphyxia	Mild Asphyxia (n=19)	Moderate (n=7)	Severe (n=3)	p-value
PAH				
Severe	1 (5.3%)	1 (14.3%)	**2 (66.7%)**	**0.047**
Moderate	4 (21.1%)	1 (14.3%)	1 (33.3%)	
Mild	**14 (73.7%)**	5 (71.4%)	0	
PDA				
Present	9 (47.4%)	1 (14.3%)	2 (66.7%)	0.203
Absent	10 (52.6%)	6 (85.7%)	1 (33.3%)	
Other congenital heart disease (DORV)				
Present	0	1 (14.3%)	1 (33.3%)	0.072
Absent	19 (100%)	6 (85.7%)	2 (66.7%)	
Ejection Fraction				
Normal EF (>=60%)	14 (73.7%)	7 (100%)	3 (100%)	0.204
Low EF	5 (26.3%)	0	0	

Figure 6: Comparison of complication among Newborns with Sarnat staging

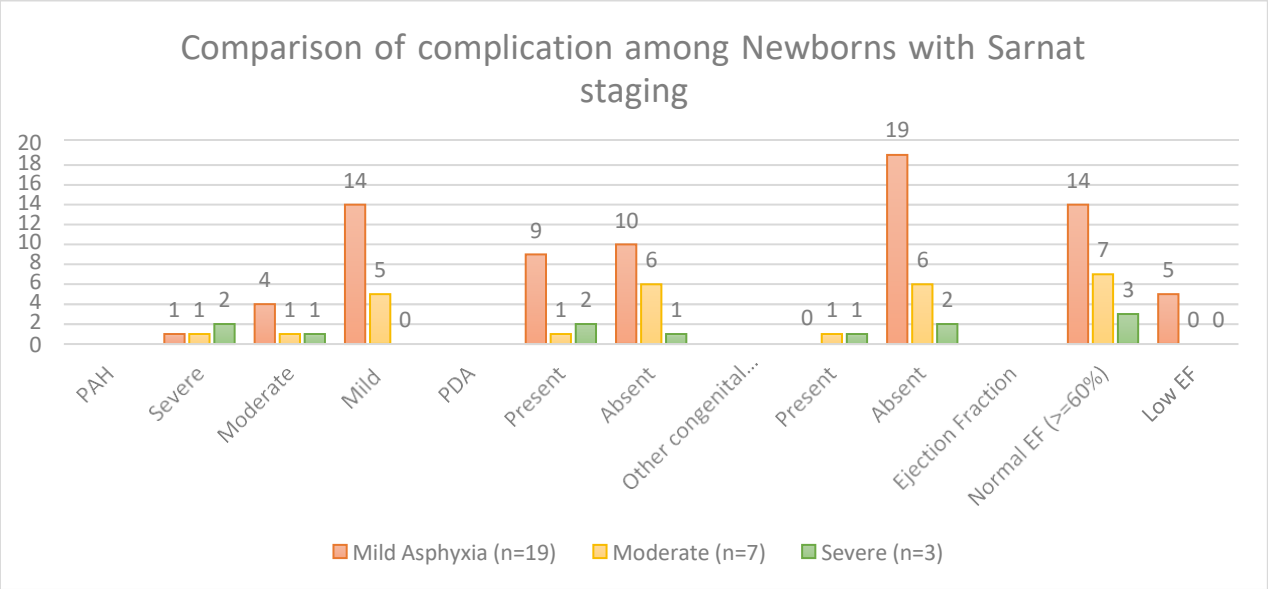


Table 5 continued: Comparison of complication among Newborns with Sarnat staging (N=102)

	Mild (n=19)	Moderate (n=7)	Severe (n=3)	p-value
Pulmonary Haemorrhage	0	2 (28.6%)	2 (66.7%)	**0.003**
Infection	4 (21.1%)	1 (14.3%)	1 (33.3%)	0.791
High FiO2 requirement (>40%)	2 (10.5%)	2 (28.6%)	2 (66.7%)	0.070
Mechanical Ventilation	1 (5.3%)	5 (71.4%)	3 (100%)	<0.001
Mean Duration of ICU stay	9.66 (5.36)	8.0 (3.11)	8.0 (7.93)	0.721
OUTCOME				
Death	1 (5.3%)	2 (28.6%)	2 (66.7%)	0.022
Alive	18 (94.7%)	5 (71.4%)	1 (33.3%)	

In our study correlation of pulmonary haemorrhage (p=0.003), PAH (0.047), were significant in comparison with stages of sarnat staging and the need of ventilator was more in moderate to severe category, the mortality was also more with moderate to severe cases.

Figure 7: Comparison of complication among Newborns with sarnat staging

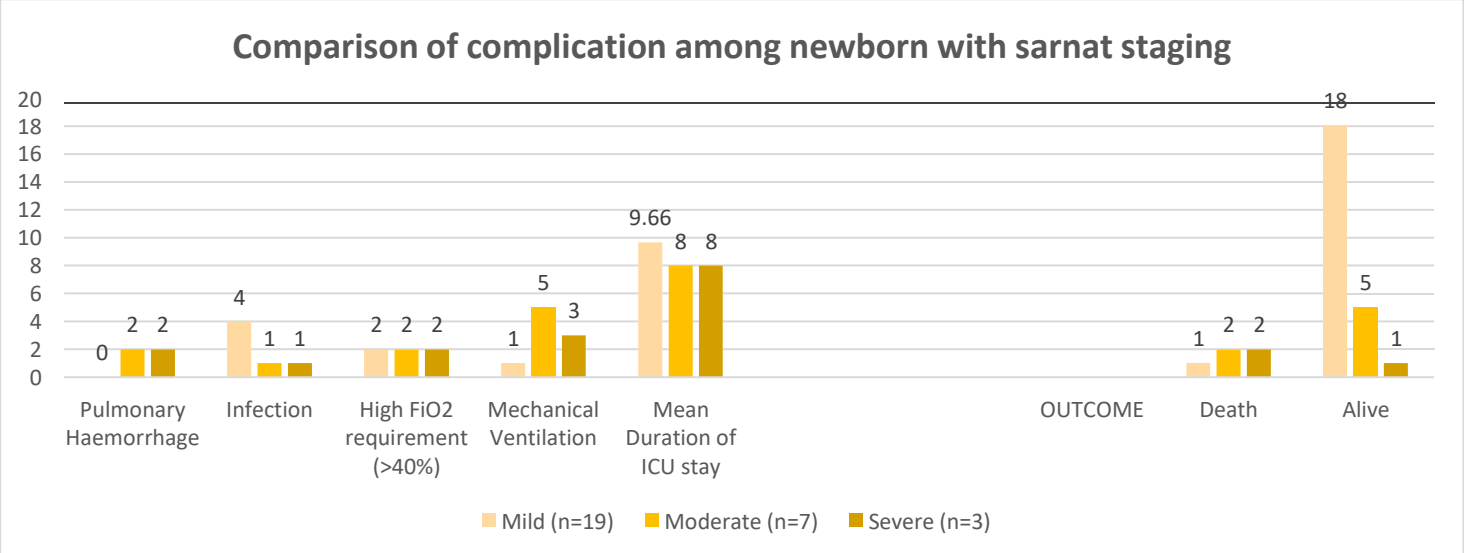


Table 6: Comparison of complication among Newborns with different Sarnat staging (N=29)

	Mild (n=19)	Moderate (n=7)	Severe (n=3)	p-value
Mean Hemoglobin	16.6 (2.3)	16.9 (1.9)	16.7 92.0)	0.966
Median (IQR) TLC	15790 (9920 – 20630)	17730 (8810 – 21180)	19270 (8810 – 23910)	0.809
Median (IQR) Platelet count	2.63 (2.05 – 2.94)	1.99 (1.78 – 2.99)	2.80 (1.71 – 3.07)	0.622
C-Reactive Protein	9.9 (5.9 – 18.1)	5.0 (<5.0 – 16.3)	5.0 (5.0 – 12.0)	0.318
Serum creatinine	0.9 (0.3)	0.9 (0.3)	1.3 (0.3)	0.088
pH categories				
6.9 – 7.1	0	1 (14.3%)	1 (33.3%)	0.085
7.1 – 7.3	5 (26.3%)	4 (57.1%)	2 (66.7%)	
7.3 – 7.45	10 (52.6%)	2 (28.6%)	0	
>7.45	4 (21.1%)	0	0	
PCO2 (35-45)	28.7 (8.9)	26.0 (10.0)	47.8 (24.7)	**0.023**
PO2	148.5 (37.7)	158.8 (56.3)	132.3 (61.3)	0.689
HCO3				
<10	2 (10.5%)	1 (14.3%)	0	0.174
10 – 15	7 (36.8%)	5 (71.4%)	3 (100%)	
>15	10 (52.6%)	1 (14.3%)	0	
Lactate	3.7 (2 – 8.4)	5.9 (4.5 – 12.3)	8.5 (8.4 – 8.6)	0.081
Mean (SD) MDA	**7.71 (1.09)**	**9.98 (2.34)**	**10.93 (2.51)**	**0.001**
Median (IQR) MDA	**8.03 (6.96 – 8.74)**	**9.83 (8.52 – 10.01)**	**10.08 (8.97 – 13.76)**	**0.004**

In our study there was no significant correlation between various laboratory parameters in sarnat stages, except PCO 2 value was significantly high in severe asphyxiated newborns ,MDA level was significantly high in HIE III (severe), compared to HIE II (moderate) and HIE I (mild)

Table 7: Correlation of maternal risk factors with low APGAR Score at 1 min. (N=29)

Pregnancy related Complications	Severe (APGAR 0-3) N=5	Moderate (APGAR 4-6) N=24	Normal (APGAR>=7) N=73	p-value
Present	2 (40.0%)	14 (58.3%)	19 (26.0%)	**0.015**
Multiple pregnancy	0	3 (12.5%)	1 (1.4%)	**0.046**
Anaemia	2 (40%)	1 (4.2%)	4 (5.5%)	**0.011**
Pre-eclampsia	1 (20.0%)	5 (20.8%)	5 (6.9%)	0.126
PROM	0	6 (25.0%)	7 (9.6%)	0.099
Placenta Previa	2 (40.0%)	5 (20.8%)	2 (2.7%)	**0.001**
History of sub-fertility	0	1 (4.2%)	2 (2.7%)	0.866
Prolonged II stage	1 (20.0%)	8 (33.3%)	5 (6.8%)	**0.004**
Gestational diabetes mellitus	0	0	4 (5.5%)	0.437
Heart disease	0	0	0	NA
UTI	0	1 (4.2%)	1 (1.4%)	0.657
Vaginitis	0	2 (8.3%)	1 (1.4%)	0.199

In our study when the maternal complications were correlated with the low APGAR scores, significant correlation was found with anaemia, placenta previa, and prolonged second stage of labour

Figure 8: Correlation of maternal risk factors with low APGAR Score at 1 min. (N=29)

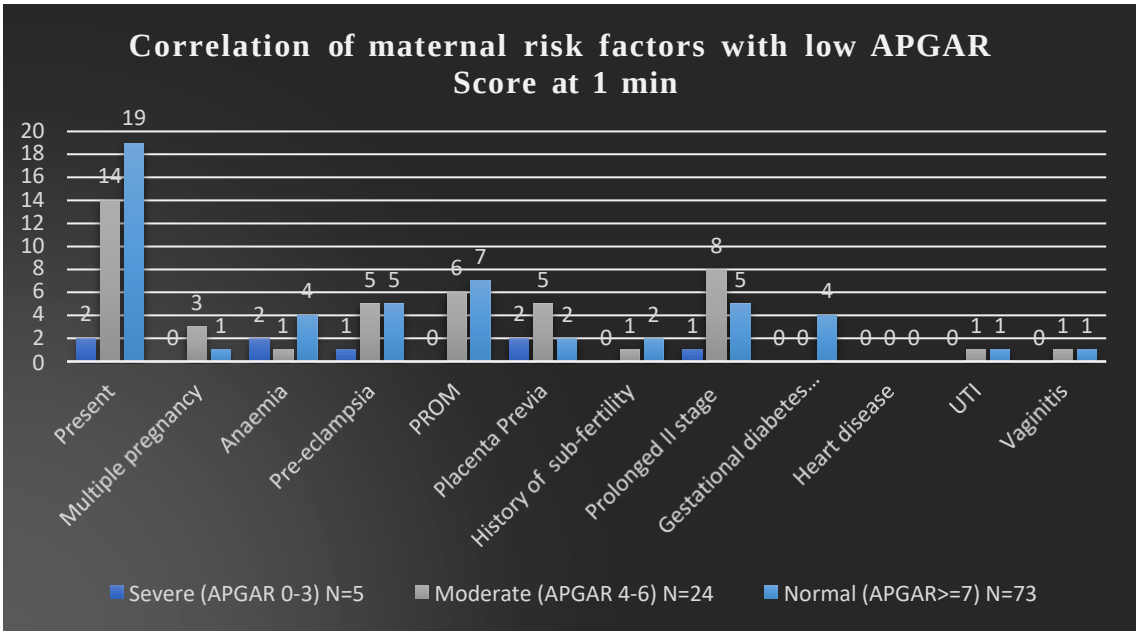


Table 8: Comparison of Maternal Risk factors, and required modes of resuscitation

Maternal risk factors	Bag and Mask (n=9)	CPR (n=3)	Intubation (n=3)	Physical stimulation (n=14)	Total (n=29)	p-value
Pregnancy related Complications	5 (55.6%)	1 (33.3%)	2 (66.7%)	8 (57.1%)	16 (55.2%)	0.859
Multiple pregnancy	2 (22.2%)	0	0	1 (7.1%)	3 (10.3%)	0.529
Anaemia	0	1 (33.3%)	2 (66.7%)	0	3 (10.3%)	**0.002**
Pre-eclampsia	2 (22.2%)	0	1 (33.3%)	3 (21.4%)	6 (20.7%)	0.779
PROM	1 (11.1%)	0	0	5 (35.7%)	6 (20.7%)	0.262
Placenta Previa	3 (33.3%)	1 (33.3%)	2 (66.7%)	1 (7.1%)	7 (24.1%)	0.126
History of subfertility	0	0	0	1 (7.1%)	1 (3.5%)	0.775
Prolonged II Stage	2 (22.2%)	1 (33.3%)	0	6 (42.9%)	9 (31.0%)	0.458
Gestational diabetes mellitus	0	0	0	0	0	NA
Heart disease	0	0	0	0	0	NA
UTI	0	0	0	1 (7.1%)	1 (3.4%)	0.775
Vaginitis	1 (11.1%)	0	0	1 (7.1%)	2 (6.9%)	0.874

Maternal risk factors	Bag and Mask (n=9)	CPR (n=3)	Intubation (n=3)	Physical stimulation (n=14)	Total (n=29)	p-value
Amniotic fluid						
Meconium	3 (33.3%)	1 (33.3%)	0	7 (50.0%)	11 (37.9%)	0.064
Blood stained	0	1 (33.3%)	0	0	1 (3.4%)	
Clear	6 (66.7%)	1 (33.3%)	3 (100%)	7 (50.0%)	17 (58.6%)	
Mode of delivery						
LSCS	5 (55.6%)	2 (66.7%)	2 (66.7%)	7 (50.0%)	16 (55.2%)	0.925
Vaginal	4 (44.4%)	1 (33.3%)	1 (33.3%)	7 (50.0%)	13 (44.8%)	
Birth weight						
1500 – 2500 gm	3 (33.3%)	1 (33.3%)	0	4 (28.6%)	8 (27.6%)	0.718
2500 – 3500 gm	6 (66.7%)	2 (66.7%)	3 (100%)	10 (71.4%)	21 (72.4%)	
Appropriate for gestation						
LGA	1 (11.1%)	0	0	0	1 (3.4%)	0.695
SGA	3 (33.3%)	1 (33.3%)	0	4 (28.6%)	8 (28.6%)	
AGA	5 (55.6%)	2 (66.7%)	3 (100%)	10 (71.4%)	20 (69.0%)	

In our study correlation of maternal risk factors with levels of resuscitation showed significance with only anemia, where as other risk factors like pre-eclampsia, placenta previa and PROM also has association but was not statistically significant

Table 9: Comparison of MDA levels among Newborns with and without birth asphyxia (N=102)

Cord blood	Newborns with Birth Asphyxia	Newborns without Birth Asphyxia	p-value
MDA micromol/L			
Mean (SD)	8.59 (1.99)	3.18 (1.04)	<0.001
Median (IQR)	8.33 (7.66 – 9.01)	2.96 (2.33 – 3.96)	<0.001

In our study the mean values of MDA was higher in newborns with asphyxia compared to those without asphyxia

Figure 9: Comparison of MDA among Newborns with and without birth asphyxia (N=102)

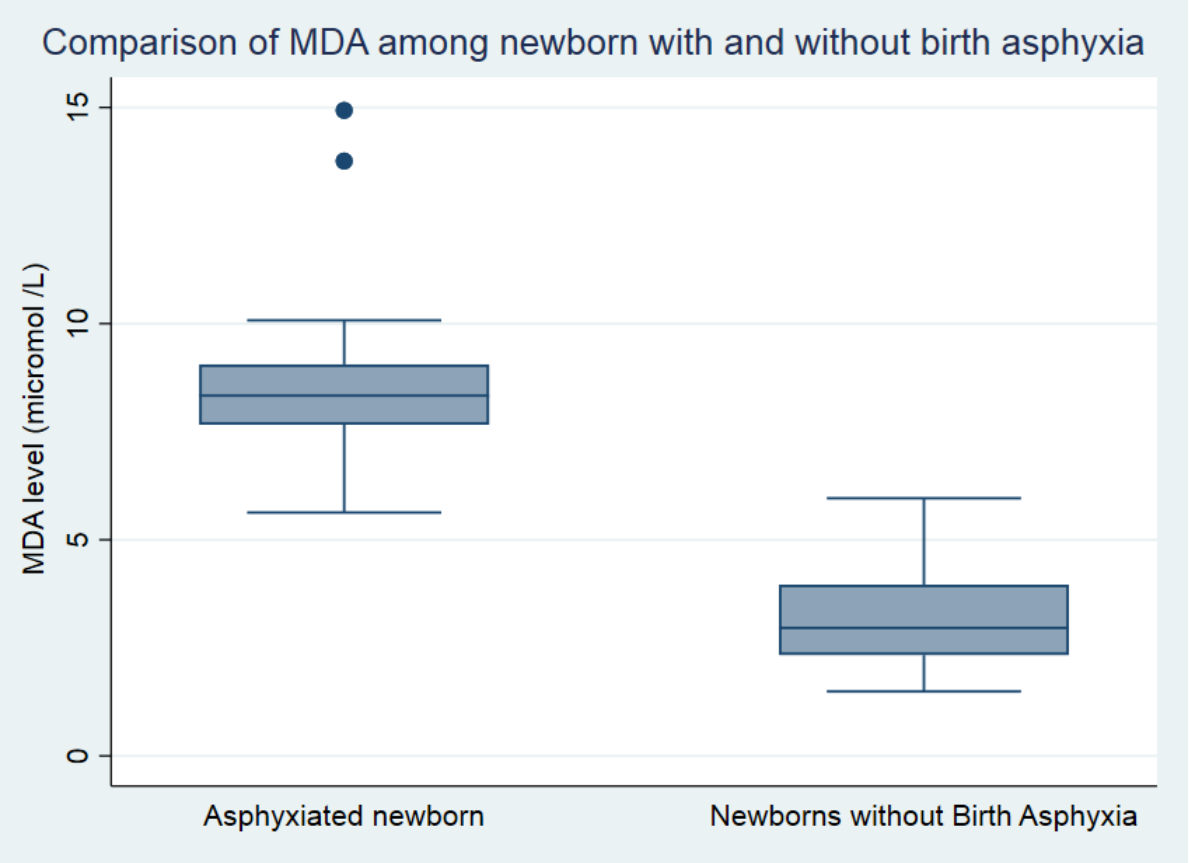


Table 10: Comparison of MDA among Newborns with different HIE Staging (N=102)

	Mean (SD)	Median (IQR)
No HIE	3.18 (1.04)	2.96 (2.33 – 3.96)
Mild	7.72 (1.09)	8.03 (6.96 – 8.74)
Moderate	9.98 (2.34)	9.83 (8.52 – 10.02)
Severe	**10.93** (2.50)	**10.08** (8.97 – 13.76)
p-value	**<0.001**	**<0.001**

In our study the cord blood mean MDA level was significantly high in HIE III (severe) ,compared to HIE II (moderate) and HIE I(mild) with a p value of <0.001

Figure 10 : Comparison of MDA among Newborns with different HIE Staging (N=102)

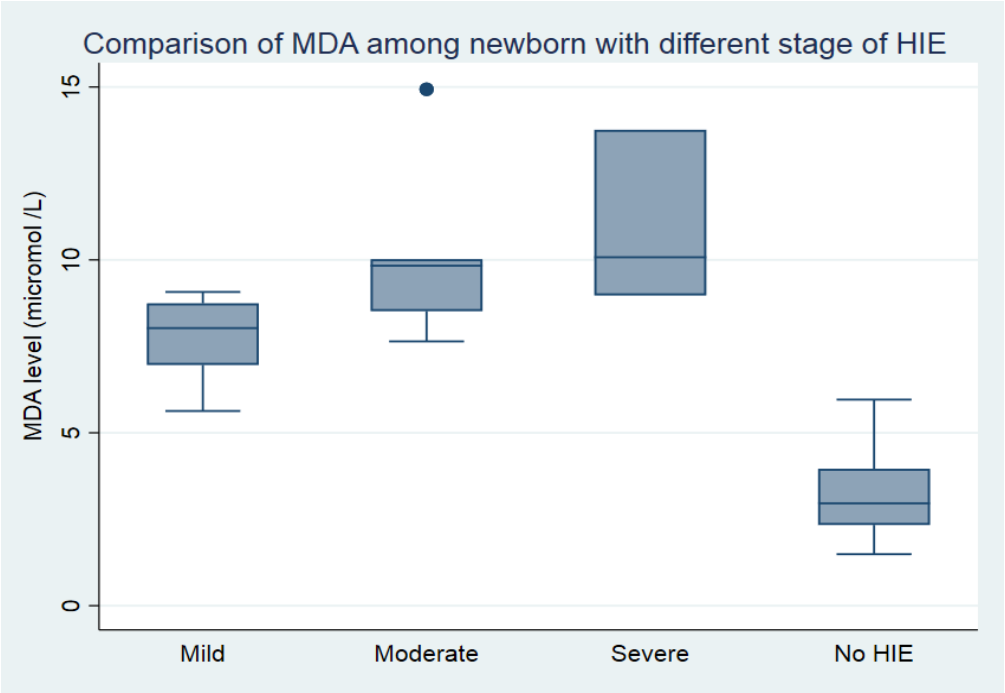


Table 11 : Comparison of MDA among Newborns requiring different modes of resuscitation (N=102)

Type of Resuscitation/MDA level	MDA level Mean (SD)	MDA Levels Median (IQR)
No resuscitation	3.18 (1.04)	2.96 (2.33 – 3.96)
Physical stimulation	7.79 (1.13)	8.07 (7.07 – 8.74)
Bag and mask	8.21 (1.19)	8.52 (7.64 – 9.02)
CPR	9.18 (1.33)	9.83 (7.66 – 10.08)
Intubation	**12.90** (2.56)	**13.76** (10.02 – 14.93)
p-value	**<0.001**	**<0.001**

In our study when cord blood MDA was compared with different levels of resuscitation , the severe cases requiring intubation and CPR showed high levels of MDA values which was statistically significant .

Figure 11 : Comparison of MDA among Newborns with different modes of resuscitation (N=102)

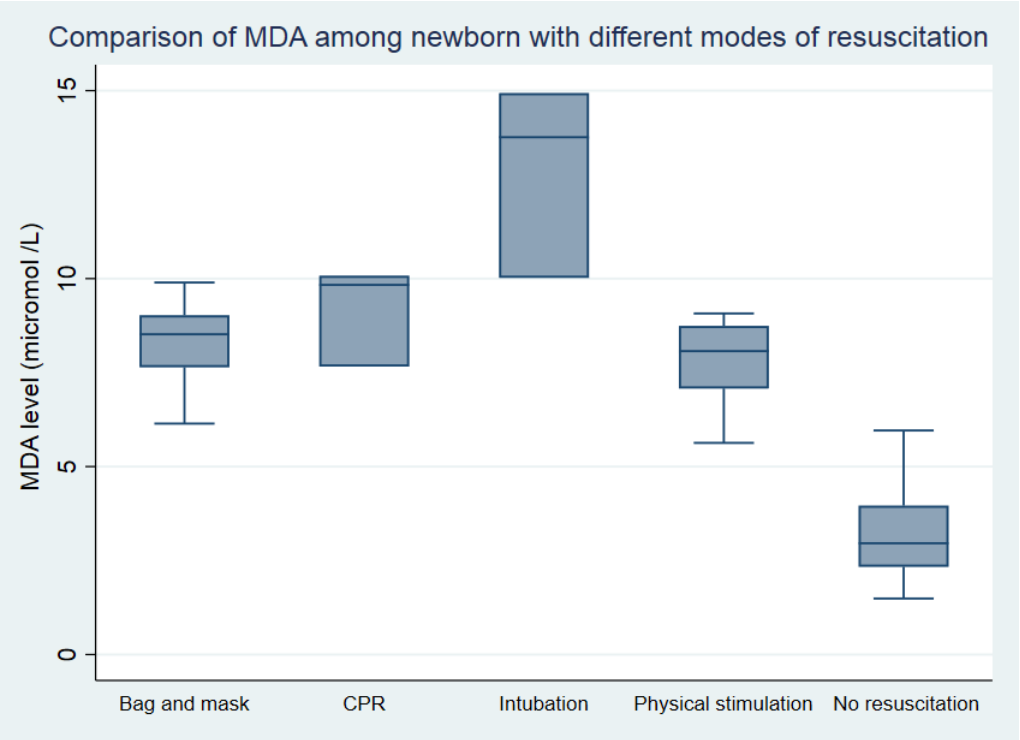


Table 12 : Comparison of MDA among Newborns who died vs who survived (N=102)

	Mean (SD)	Median (IQR)
Died	**10.87** (3.24)	**9.07** (8.97 – 13.760
Survived	4.40 (2.40)	3.68 (2.53 – 5.63)
p-value	**<0.001**	**<0.001**

In our study we have observed the mean MDA level was high in newborn who were dead than those who survived

Figure 12 : Comparison of MDA among Newborns who died vs who survived (N=102)

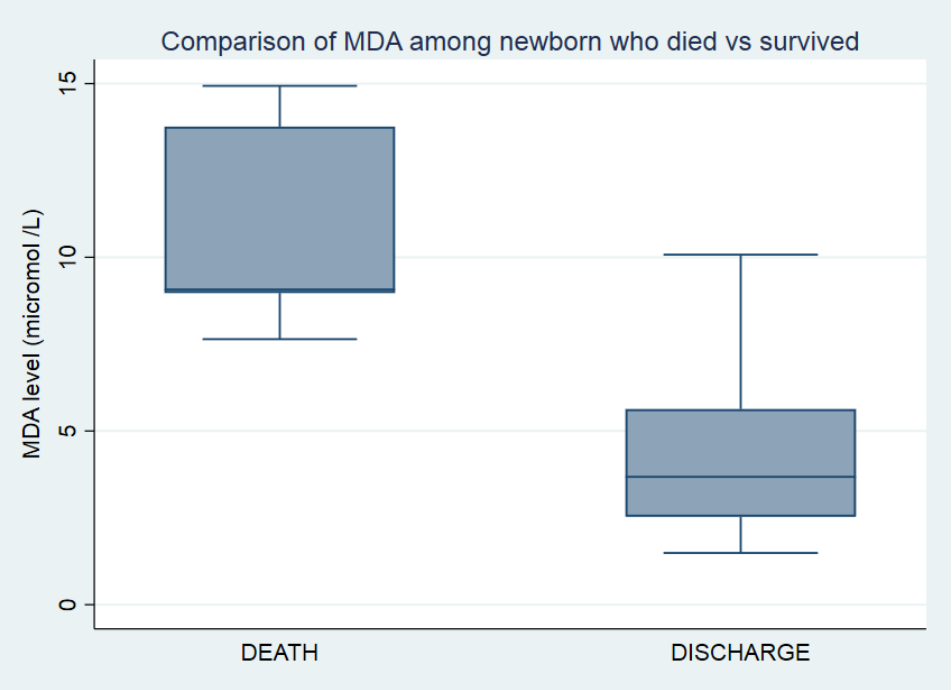
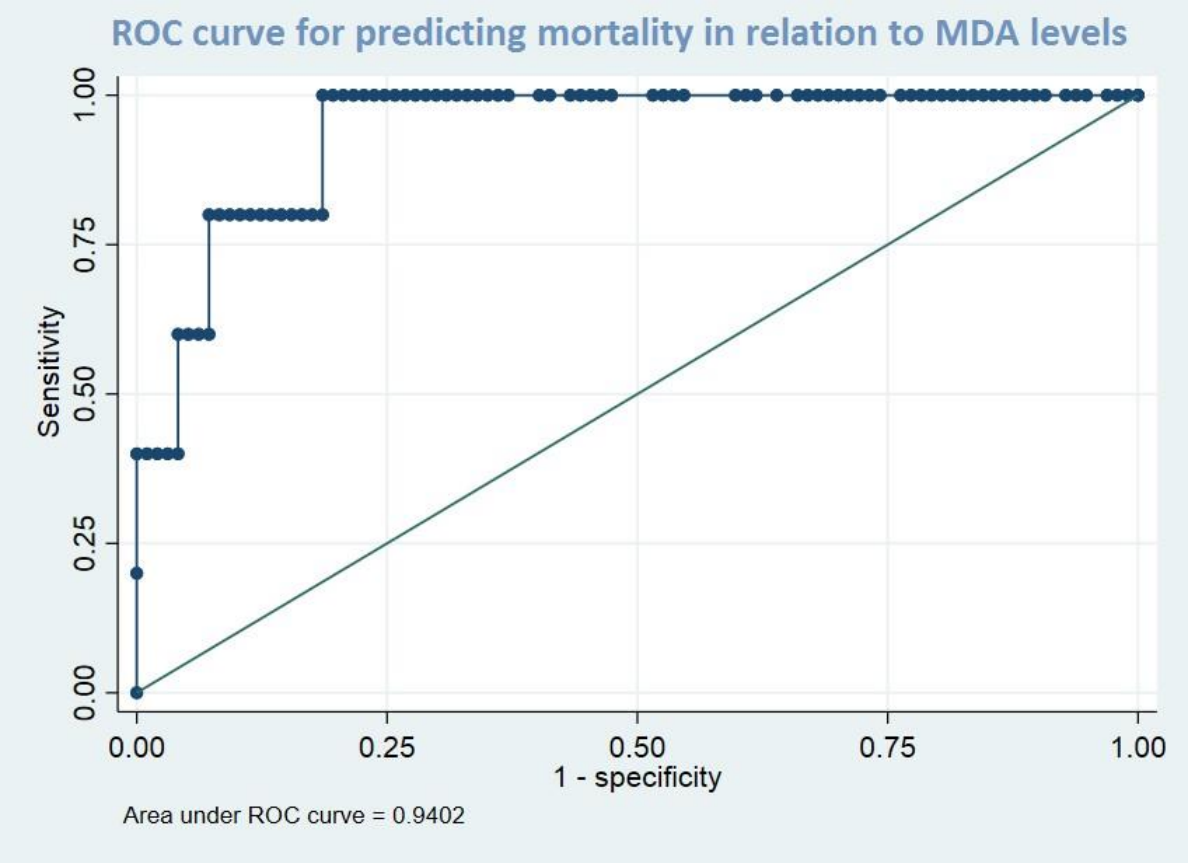


Table 13: ROC curve for predicting mortality among newborn with Birth Asphyxia in relation to MDA levels

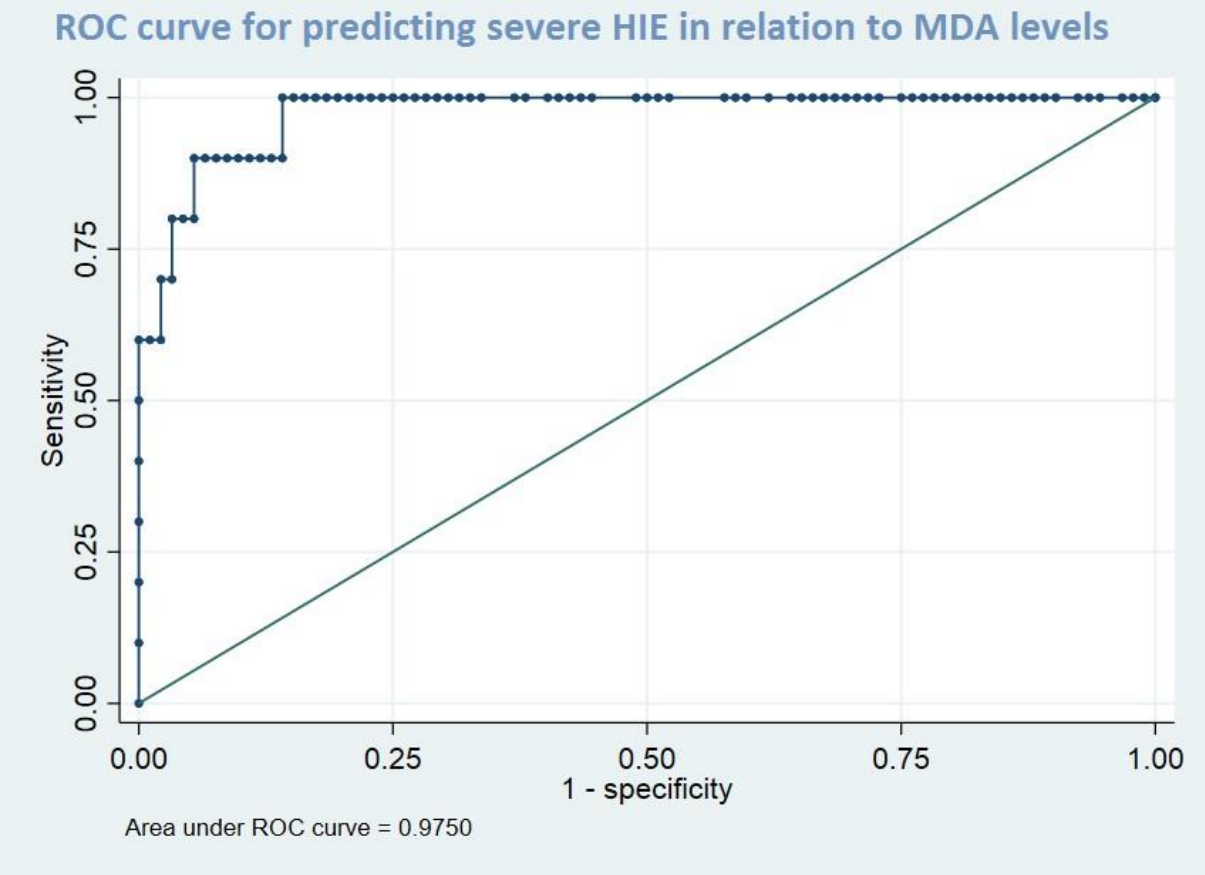
	Mortality	Severe-moderate staging of HIE
ROC – Area under the curve	0.940	0.975
Standard Error	0.037	0.016
95% CI	0.867 – 1.00	0.943 – 1.00
Cut-off	7.64	7.64
Sensitivity	100%	100%
Specificity	81.4%	85.9%
Accuracy	82.35%	86.27%

Figure 13 : ROC curve for predicting mortality among newborn in relation to MDA levels



Receiver operating characteristic (ROC) curves were drawn for MDA levels to predict mortality and severe HIE by plotting sensitivity versus one minus specificity. The area under the receiver operating characteristic curve (AUC) was used to evaluate the discriminatory capacity of the MDA levels to predict mortality. The ROC curve for predicting mortality among newborns showed high predictive accuracy of 82.35% with good discriminatory power. The area under the curve was 0.940 (95% CI: 0.867 – 1.00). The cut-off of 7.64 had a sensitivity of 100% and specificity of 81.4%.

Figure 13 : ROC curve for predicting staging in HIE in relation to MDA levels



The area under the receiver operating characteristic curve (AUC) was used to evaluate the discriminatory capacity of the MDA levels. The ROC curve for predicting severe HIE among

newborns showed high predictive accuracy of 86.27% with good discriminatory power. The area under the curve was 0.975 (95% CI: 0.943 – 1.00). The cut-off of 7.64 had a sensitivity of 100% and specificity of 85.9%.

Table 16: Outcome in different groups

Characteristics	DIED (n=5)	DISCHARGED (n=97)	p-value
BIRTH ASPHYXIA			
Newborns with Birth Asphyxia	5 (17.2%)	24 (82.8%)	**<0.001**
Normal Newborns	0	73 (100%)	
HIE Staging			
Mild	1 (5.3%)	18 (94.7%)	**0.022**
Moderate	2 (28.6%)	5 (71.4%)	
Severe	2 (66.7%)	1 (33.3%)	
Mode of resuscitation			
Physical stimulation	1 (7.1%)	13 (92.9%)	**0.075**
Bag and mask	2 (22.2%)	7 (77.8%)	
CPR	0	3 (100%)	
Intubation	2 (66.7%)	1 (33.3%)	
APGAR at birth			
Severe (APGAR <=3)	1 (20.0%)	4 (80.0%)	**0.001**
Moderate (APGAR 4-6)	4 (16.7%)	20 (83.3%)	
Normal (APGAR>7)	0	73 (100%)	

In this study there was a significant correlation with outcomes of neonate and with HIE staging, low APGAR scores. The mortality was high in newborns with low APGAR scores and severe birth asphyxia

DISCUSSION

According to the National Neonatal Forum of India, birth asphyxia should be suspected when a new born breathes insufficiently or not at all after one minute." It is an efficient definition that can be applied in the community. Gasping or lack of breathing at 1 minute is an appropriate definition for purposes of estimating the occurrences of birth asphyxia and corresponds to a 1-minute Apgar score of 3 or below. Additionally, the Apgar score at 5 and 10 minutes of age and the degree of hypoxia were rated. The community and other hospitals with compromised facilities can adopt the NNF concept because it is straightforward and easy to implement. Many perinatal deaths occur with birth asphyxia, which affects newborns of women who are referred after experiencing potentially fatal obstetric problems, Ours is a Tertiary care Centre receiving referrals from the peripheries.

In our study we have observed a significant correlation between various maternal factors with relation to birth asphyxia, Pre-eclampsia in 6 (54.5%) newborns with birth asphyxia with p value-0.042), placenta previa in 7(77.8%, P=0.001), prolonged second stage (>2hours) in 9(64.3% ,p=0.001) , and Meconium stained amniotic fluid in11(57.9%, P=0.001) of asphyxiated newborns .Gestational Diabetes Mellitus was same in both the asphyxiated and normal newborns in our study

Similar results were seen in a retrospective study conducted by Yi Yu et al..[57]it is a large cohort multicentre study they have observed the meconium amniotic fluid was widely identified as a risk factor for Neonatal Asphyxia (NA). Mothers with hypertensive disorders (11.6% in NA $P < 0.001$), placenta previa (6.6% in NA $P < 0.001$), were more likely to lead to NA. The presences of gestational diabetes mellitus were similar in the two groups (13.0% in NA, $P = 0.445$), However, the occurrences

of pre-existing diabetes mellitus and anemia weren't significantly higher in the NA group. Premature rupture of membranes (PROM) was presented in 210(18.2%) of the NA ($P < 0.001$).

In the same study the neonates complicated with asphyxia tended to be smaller in gestational week and birth weight , similarly in our study low birth weight was seen in 80% and Term SGA newborns were 80% showing significance with p=<0.001

Requirement of Mechanical ventilation was more in severely asphyxiated newborn (p=0.001), with mortality in 5 newborns (4 moderate to severely asphyxiated, 1 mild asphyxia with sepsis)

In a study by Pradhan SK et al..,[58]maternal age below 20 years, primigravida status, maternal anemia(p value <0.001) and sub-optimal antenatal care are significant risk factors for HIE. Similar results were found in our study with significant correlation of maternal anemia with low APGAR scores($p<0.001$).

In our study 102 mothers were selected randomly to assess the impact of pregnancy related complications on the levels of resuscitation required in the newborns and its outcome, we analysed Pregnancy related complications, 29 newborns required different levels of resuscitation, the major impact was observed due to severe Maternal anemia associated with placenta previa required higher levels of resuscitation , there 7 newborns with maternal h/o placenta previa requiring resuscitation but it was not statistically significant. In our study, we observed that multiple maternal risk factors contributes to requirement of advanced levels of resuscitation at birth resulting in birth asphyxia. Hence birth asphyxia is associated with multifactorial causes secondary to maternal comorbidities during pregnancy.

In a study by Shahrzad Tabatabaee et al..[59]the evaluation of mothers, 117 (60.6%) subjects had medical problems during pregnancy they showed, the most prevalent problem was preeclampsia in 44 (22.8%) subjects. In the follow-up of the patients, 99 (51.3%) and 127 (65.8%) neonates needed mechanical ventilation within 24 h of life and surfactant administration via endotracheal intubation, respectively. They showed that the level of resuscitation has a significant effect on the rate of neonatal mortality (P<0.001), and mortality due to hypoxic-ischemic encephalopathy (HIE) has a statistical difference in higher levels of resuscitation.

The rate of neonatal mortality in our population was 23.8% (n=46), and HIE was recorded in 21.7% (n=10) of dead neonates. The mean duration of hospital stay in neonates was 29.9+22.7 days

In our study we have assessed serum MDA level in cord blood and we have found significant difference in normal and asphyxiated newborns with cord blood MDA levels of **8.59 *(*1.99)** in asphyxiated and **3.18 (1.04)** in normal newborns, **(P<0.001),** we have also found significant difference in cord blood MDA levels in respect to different levels of resuscitation, with increasing values in higher levels like **Intubation-9.18 (1.33) and CPR -12.90 (2.56).** we have also found, and increasing levels with low APGAR and that deceased had higher cord MDA levels than those who survived.

In a study by Nivedita Mondal et al..[54]The mean MDA (in micromoles/L) and protein carbonyl at birth were found to be significantly higher in cases5.88 ± 1.401 and in controls (normal newborn) 3.11 ± 0.82. In the study group the values at 48 hours were significantly higher than those at birth.

The mean value of MDA at birth was found to increase with worsening stages of HIE,with HIE III having a significantly higher value as compared to HIE I and HIE II. However, MDA at 48 hours and protein carbonyl values though progressively rose in higher stages of HIE, the differences were not found to be statistically significant, In our study we did not assess protein carbonyl and 48 hours values of MDA

In our study cord MDA level for neonates with Sarnat's grade I was significantly lower than for neonates with grades II and III in our study, which was graded according to Sarnat's scoring. The level of MDA detected in grade III newborns was higher than that observed in grade II neonates with MDA values [in micromoles /L], and there was a significant difference between the cord MDA levels for neonates with grades II and III. Grades I and II HIE are 7.72 (1.09), 9.98 (2.34), and 10.93 respectively (2.50) This method may be useful to assess the prognosis of newborns with HIE because the results revealed a substantial association between the cord MDA level and the amount of cerebral injury (as determined by Sarnat's grading).

In a study by E KIRIMI et al.,[60] the cord MDA level for infants with Sarnat's grade I was considerably lower than for neonates with grades II and III. The association between the severity of clinical symptoms and the cord MDA level was also examined in neonates with HIE. Although the cord MDA level for neonates in grades II and III did not significantly differ from one another, the amount observed in grade III neonates was higher than that reported in grade II neonates. Malondialdehyde

levels were (nmol/ml or micromoles/L), mean ± SD 3.46 ± 0.95 in Grade I HIE, 6.47 ± 1.21 in Grade II HIE, 7.72 ± 2.82 in Grade III HIE

The correlation of maternal risk factors and MDA level in cord blood is new correlation done in this study and it was statistically significant

CONCLUSION

1.Newborns with high oxidative stress due to maternal or fetal risk factors and low APGAR scores needed advanced resuscitation .

2. Poor Neonatal outcome in low APGAR babies is associated with the high values of MDA.

MDA levels above 7.64 micromoles/L have100% sensitivity and 81.4% specificity with respect to Mortality in asphyxiated Newborns, 85.9% with HIE staging (moderate-severe)

3. Hence cord blood MDA can be a marker to predict the outcome in asphyxiated newborns.

Limitations:

1.Small cohort of cases in moderate and severe asphyxia

2. subjective assessment of APGAR scoring

Further studies may lead to use of antioxidants, and modifying maternal risk factors to reduce Neonatal asphyxia. And precisely this is what we want to happen for better neonatal outcome.

BIBLIOGRAPHY

1. Escobedo MB, Aziz K, Kapadia VS, Lee HC, Niermeyer S, Schmölzer GM, et al. 2019 American Heart Association Focused Update on Neonatal Resuscitation: An Update to the American Heart Association Guidelines for Cardiopulmonary Resuscitation and Emergency Cardiovascular Care. Circulation. 2019 Dec 10;140(24):e922–30.

2. Bryce J, Boschi-Pinto C, Shibuya K, Black RE. WHO estimates of the causes of death in children. Lancet.2005 Mar 26-Apr 1. 365(9465):1147-52.[Medline].

3. Lawn J, Shibuya K, Stein C. No cry at birth: global estimates of intrapartum stillbirths and intrapartum-related neonatal deaths. Bull World Health Organ. 2005 Jun.83(6):409-17.

4. Tabatabaee S, Afjeh A, Radfar M, Fallahi M. Assessment of delivery room resuscitation with different levels and its related factors in preterm neonates. Iranian Journal of Neonatology IJN. 2020;11(3):57-63.

5. Martin RJ, Fanaroff AA, Walsh MC. Fanaroff and Martin's neonatal-perinatal medicine e-book: diseases of the fetus and infant. Elsevier Health Sciences; 2014 Aug 20.

6. Nicolaides KH, Economides DL, Soothill PW. Blood gases, pH, and lactate in appropriate- and small-for-gestational-age fetuses. American Journal of Obstetrics & Gynecology. 1989 Oct 1;161(4):996–1001.

7. Mir IN, Johnson-Welch SF, Nelson DB, Brown LS, Rosenfeld CR, Chalak LF. Placental pathology is associated with severity of neonatal encephalopathy and adverse developmental

outcomes following hypothermia. American journal of obstetrics and gynecology. 2015 Dec 1;213(6):849-e1.

8. Rudolph AM, Heymann MA. The circulation of the fetus in utero: methods for studying distribution of blood flow, cardiac output and organ blood flow. Circulation research. 1967 Aug;21(2):163-84.

9. Rudolph AM, Yuan S. Response of the pulmonary vasculature to hypoxia and H+ ion concentration changes. The Journal of clinical investigation. 1966 Mar 1;45(3):399-411.

10. Shah P, Riphagen S, Beyene J, Perlman M. Multiorgan dysfunction in infants with post-asphyxial hypoxic-ischaemic encephalopathy. Archives of Disease in Childhood-Fetal and Neonatal Edition. 2004 Mar 1;89(2):F152-5.

11. Phelan JP, Ahn MO, Korst L, Martin GI, Wang YM. Intrapartum fetal asphyxial brain injury with absent multiorgan system dysfunction. The Journal of Maternal-Fetal Medicine. 1998 Jan;7(1):19-22.

12. Low JA, Galbraith RS, Muir DW, Killen HL, Pater EA, Karchmar EJ. Factors associated with motor and cognitive deficits in children after intrapartum fetal hypoxia. American journal of obstetrics and gynecology. 1984 Mar 1;148(5):533-9.

13. Pasternak JF, Gorey MT. The syndrome of acute near-total intrauterine asphyxia in the term infant. Pediatric neurology. 1998 May 1;18(5):391-8.

14. Cremer JE. Substrate utilization and brain development. Journal of Cerebral Blood Flow & Metabolism. 1982 Dec;2(4):394-407.

15. Wimberley PD. A review of oxygen and delivery in the neonate. Scand J Clin Lab Invest. 1982;160(Suppl):114-8.

16. Aberman A. Crossover PO2, a measure of the variable effect of increased P50 on mixed venous PO2. American Review of Respiratory Disease. 1977 Jan;115(1):173-5.

17. Woodson RD. Physiological significance of oxygen dissociation curve shifts. Critical Care Medicine. 1979 Sep 1;7(9):368-73.

18. Cowett RM, editor. Principles of perinatal-neonatal metabolism. Springer Science & Business Media; 2012 Dec 6.

19. Thorp JA, Rushing RS. Umbilical cord blood gas analysis. Obstetrics and gynecology clinics of North America. 1999 Dec 1;26(4):695-709.

20. Goldaber KG, Gilstrap 3rd LC, Leveno KJ, Dax JS, McINTIRE DD. Pathologic fetal acidemia. Obstetrics and gynecology. 1991 Dec 1;78(6):1103-7.

21. Sehdev HM, Stamilio DM, Macones GA, Graham E, Morgan MA. Predictive factors for neonatal morbidity in neonates with an umbilical arterial cord pH less than 7.00. American journal of obstetrics and gynecology. 1997 Nov 1;177(5):1030-4.

22. Perlman JM, Risser R. Severe fetal acidemia: neonatal neurologic features and short-term outcome. Pediatric neurology. 1993 Jul 1;9(4):277-82.

23. Goodwin TM, Belai I, Hernandez P, Durand M, Paul RH. Asphyxial complications in the term newborn with severe umbilical acidemia. American journal of obstetrics and gynecology. 1992 Dec 1;167(6):1506-12.

24. Fee SC, Malee K, Deddish R, Minogue JP, Socol ML. Severe acidosis and subsequent neurologic status. American journal of obstetrics and gynecology. 1990 Mar 1;162(3):802-6.

25. Calvert JW, Zhang JH. Pathophysiology of an hypoxic–ischemic insult during the perinatal period. Neurological research. 2005 Apr 1;27(3):246-60.

26. Northington FJ, Chavez-Valdez R, Martin LJ. Neuronal cell death in neonatal hypoxia-ischemia. Annals of neurology. 2011 May;69(5):743-58.

27. Vannucci RC, Towfighi J, Vannucci SJ. Secondary energy failure after cerebral hypoxia–ischemia in the immature rat. Journal of Cerebral Blood Flow & Metabolism. 2004 Oct;24(10):1090-7.

28. Vannucci RC, Yager JY. Glucose, lactic acid, and perinatal hypoxic-ischemic brain damage. Pediatric neurology. 1992 Jan 1;8(1):3-12.

29. Dixon BJ, Reis C, Ho WM, Tang J, Zhang JH. Neuroprotective strategies after neonatal hypoxic ischemic encephalopathy. International journal of molecular sciences. 2015 Sep 15;16(9):22368-401.

30. Kasdorf E, Engel M, Heier L, Perlman JM. Therapeutic hypothermia in neonates and selective hippocampal injury on diffusion-weighted magnetic resonance imaging. Pediatric Neurology. 2014 Jul 1;51(1):104-8.

31. Wyckoff MH, Aziz K, Escobedo MB, Kapadia VS, Kattwinkel J, Perlman JM, Simon WM, Weiner GM, Zaichkin JG. Part 13: neonatal resuscitation: 2015 American Heart Association

guidelines update for cardiopulmonary resuscitation and emergency cardiovascular care. Circulation. 2015 Nov 3;132(18_suppl_2):S543-60.

32. Perlman JM. Intrapartum asphyxia and cerebral palsy: is there a link?. Clinics in perinatology. 2006 Jun 1;33(2):335-53.

33. Kasdorf E, Grunebaum A, Perlman JM. Subacute hypoxia-ischemia and the timing of injury in treatment with therapeutic hypothermia. Pediatric neurology. 2015 Nov 1;53(5):417-21.

34. Rutherford M, Counsell S, Allsop J, Boardman J, Kapellou O, Larkman D, Hajnal J, Edwards D, Cowan F. Diffusion-weighted magnetic resonance imaging in term perinatal brain injury: a comparison with site of lesion and time from birth. Pediatrics. 2004 Oct;114(4):1004-14.

35. Barkovich AJ, Miller SP, Bartha A, Newton N, Hamrick SE, Mukherjee P, Glenn OA, Xu D, Partridge JC, Ferriero DM, Vigneron DB. MR imaging, MR spectroscopy, and diffusion tensor imaging of sequential studies in neonates with encephalopathy. American Journal of Neuroradiology. 2006 Mar 1;27(3):533-47.

36. Ferriero DM. Oxidant Mechanisms in Neonatal Hypoxia-Ischemia. DNE. 2001;23(3):198–202.

37. Gonzalez FF, Ferriero DM. Neuroprotection in the Newborn Infant. Clinics in Perinatology. 2009 Dec 1;36(4):859–80.

38. Ferriero DM, Holtzman DM, Black SM, Sheldon RA. Neonatal mice lacking neuronal nitric oxide synthase are less vulnerable to hypoxic–ischemic injury. Neurobiology of disease. 1996 Feb 1;3(1):64-71.

39. Ergenekon E, Gücüyener K, Erbaş D, Ezgü FS, Atalay Y. Cerebrospinal fluid and serum nitric oxide levels in asphyxiated newborns. Neonatology. 1999;76(4):200-6.

40. Groenendaal F, Vles J, Lammers H, De Vente J, Smit D, Nikkels PG. Nitrotyrosine in human neonatal spinal cord after perinatal asphyxia. Neonatology. 2008;93(1):1-6.

41. Gitto E, Reiter RJ, Cordaro SP, La Rosa M, Chiurazzi P, Trimarchi G, Gitto P, Calabró MP, Barberi I. Oxidative and inflammatory parameters in respiratory distress syndrome of preterm newborns: beneficial effects of melatonin. American journal of perinatology. 2004 May;21(04):209-16.

42. Fulia, F.; Gitto, E.; Cuzzocrea, S.; Dugo, L.; Gitto, P.; Barberi, S.; Cordaro, S.; Barberi, I. Increased levels of malondialdehyde and nitrite/nitrate in the blood of asphyxiated newborns: Reduction by melatonin. J. Pineal Res. 2001, 31, 343–349

43. Foster-Barber A, Ferriero DM. Neonatal encephalopathy in the term infant: Neuroimaging and inflammatory cytokines. Mental Retardation and Developmental Disabilities Research Reviews. 2002;8(1):20–4.

44. Hagberg H, Mallard C, Ferriero DM, Vannucci SJ, Levison SW, Vexler ZS, et al. The role of inflammation in perinatal brain injury. Nat Rev Neurol. 2015 Apr;11(4):192–208.

45. Wu YW, Escobar GJ, Grether JK, Croen LA, Greene JD, Newman TB. Chorioamnionitis and Cerebral Palsy in Term and Near-Term Infants. JAMA. 2003 Nov 26;290(20):2677–84.

46. Jenster M, Bonifacio SL, Ruel T, Rogers EE, Tam EW, Partridge JC, et al. Maternal or neonatal infection: association with neonatal encephalopathy outcomes. Pediatr Res. 2014 Jul;76(1):93–9.

47. Harteman JC, Nikkels PGJ, Benders MJNL, Kwee A, Groenendaal F, de Vries LS. Placental Pathology in Full-Term Infants with Hypoxic-Ischemic Neonatal Encephalopathy and Association with Magnetic Resonance Imaging Pattern of Brain Injury. The Journal of Pediatrics. 2013 Oct 1;163(4):968-975.e2.

48. Khanna K, Nikunji N, Khanna HD. A study of antioxidant and their preventive potential in cervical dysplasia. Asian J Obs Gynae Pract 2002;l6:3-11.

49. Choudhari SK, Chaudhary M, Gadbail AR, Sharma A, Tekade S. Oxidative and antioxidative mechanisms in oral cancer and precancer: a review. Oral Oncol. 2014; 50:10-18.

50. Zhang Y, Chen SY, Hsu T, Santella RM (2002). Immunohistochemical detection of malondialdehyde-DNA adducts in human oral mucosa cells. Carcinogenesis, 23: 207-211.

51. Mateos R, Goya L, Bravo L (2004). Determination of malondialdehyde by liquid chromatography as the 2, 4-dinitrophenylhydrazone derivative: a marker for oxidative stress in cell cultures of human hepatoma HepG2. J Chromatogr B Analyt Technol Biomed Life Sci, 805: 33- 39.

52. Korchazhkina O, Exley C, Andrew, SS (2003). Measurement by reversed-phase high performance liquid chromatography of malondialdehyde in normal human urine following

derivatisation with 2,4-dinitrop- henylhydrazine. J Chromatogr B Analyt Technol Biomed Life Sci, 794: 353-362.

53. Kumar A, Ramakrishna S, Basu S, Rao G. Oxidative Stress in Perinatal Asphyxia. Pediatric neurology. 2008 Apr 1;38:181–5.

54. Mondal N, Bhat B, Newton B, Koner B. Oxidative stress in perinatal asphyxia in relation to outcome. Indian journal of pediatrics. 2010 May 1;77:515–7.

55. Sarnat HB, Sarnat MS. Neonatal encephalopathy following fetal distress: a clinical and electroencephalographic study. Arch Neurol 1976;33:696-705.

56. Buege JA, Aust SD. [30] Microsomal lipid peroxidation. InMethods in enzymology 1978 Jan 1 (Vol. 52, pp. 302-310). Academic press.

57. Yu Y, Gao J, Liu J, Tang Y, Zhong M, He J, Liao S, Wang X, Liu X, Cao Y, Liu C. Perinatal maternal characteristics predict a high risk of neonatal asphyxia: A multi-center retrospective cohort study in China. Frontiers in medicine. 2022;9.

58. Pradhan SK, Nanda AK, Alada LM. Maternal & Fetal Risk Factors for Hypoxic-ischemic Encephalopathy (HIE) in Newborns: A Case-control Study From Southern Odisha, India. International Journal of Health Sciences.(II):13076-82.]

59 . Tabatabaee S, Afjeh A, Radfar M, Fallahi M. Assessment of delivery room resuscitation with different levels and its related factors in preterm neonates. Iranian Journal of Neonatology IJN. 2020;11(3):57-63.

60. Kirimi E, Peker E, Tuncer O, Yapicioglu H, Narli NE, Satar ME. Increased serum malondialdehyde level in neonates with hypoxic-ischaemic encephalopathy: prediction of disease severity. Journal of International Medical Research. 2010 Feb;38(1):220-6.

ANNEXURE -I

B.L.D.E. (DEEMED TO BE UNIVERSITY) IEC/No-09/2021 Date-22/01/2021

(Declared vide notification No. F.9-37/2007-U.3 (A) Dated. 29-2-2008 of the MHRD, Government of India under Section 3 of the UGC Act, 1956)

The Constituent College

SHRI. B. M. PATIL MEDICAL COLLEGE, HOSPITAL AND RESEARCH CENTRE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Institutional ethical committee of this college met on 11-01-2021 at 11-00 am to scrutinize the synopsis of Postgraduate students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected and revised version synopsis of the Thesis has been accorded Ethical Clearance

Title: Outcome of asphyxiated newborn, correlation with maternal factors and to level of oxidative stress in the newborn

Name of PG student: Dr Pidikiti Lavanya , Department of Paediatrics

Name of Guide/Co-investigator: Dr R H Gobbur, Professor of Paediatrics

DR .S.V.PATIL
CHAIRMAN, IEC
Institutional Ethical Committee
B L D E (Deemed to be University)
Shri B.M. Patil Medical College,
VIJAYAPUR-586103 (Karnataka)

Following documents were placed before Ethical Committee for Scrutinization:

1. Copy of Synopsis / Research project
2. Copy of informed consent form
3. Any other relevant documents.

8

ANNEXURE -II

RESEARCH INFORMED CONSENT FORM

BLDEA's Shri B.M. PATIL Medical College, Hospital & Research Centre,

Vijayapura, Karnataka -586103.

TITLE OF THE PROJECT

"OUTCOME OF ASPHYXIATED NEWBORN, CORRELATION WITH MATERNAL FACTORS AND TO LEVEL OF OXIDATIVE STRESS IN THE NEWBORN"

GUIDE: DR. R. H. GOBBUR, MD

PROFESSOR,

DEPARTMENT OF PEDIATRICS

PG STUDENT -Dr. PIDIKITI LAVANYA

PURPOSE OF RESEARCH:

I understand that, the present study is being carried out, to help, assess the clinical profile of all neonates and Asphyxiated newborns, compare the level of resuscitation and oxidative marker levels.

PROCEDURE:

I understand that after having obtained a detailed clinical history, thorough clinical examination and relevant investigations, a final work up of the procedure and its outcome is planned.

RISK AND DISCOMFORTS:

I understand that I may experience some pain and discomforts during the examination or my

treatment. This is mainly the result of my condition and the procedures of this study are not expected to exaggerate these feelings, which are associated with the usual course of treatment.

BENEFITS:

I understand that my participation in the study will have no direct benefit to me other than the potential benefit of the treatment.

CONFIDENTIALITY:

I understand that the medical information produced by this study will become a part of hospital records and will be subject to confidentiality. Information of sensitive personal nature will not be part of the medical record but will be stored in the investigations research file. If the data are used for publication in the medical literature or for teaching purpose, no name will be used and other identifiers such as photographs will be used only with special written permission. I understand that I may see the photograph before giving permission.

REQUEST FOR MORE INFORMATION:

I understand that I may ask more questions about the study at any time; Dr. Pidikiti Lavanya, at the department of pediatrics, is available to answer my questions or concerns. I understand that I will be informed of any significant new findings discovered during the course of the study, which might influence my continued participation. A copy of this consent form will be given to me to keep for careful reading.

REFUSAL FOR WITHDRAWAL OF PARTICIPATION:

I understand that my participation is voluntary and that I may refuse to participate or may withdraw consent and discontinue participation in the study at any time without prejudice. I also understand that Dr Pidikiti Lavanya may terminate my participation in the study after he/she has explained the reasons for doing so.

INJURY STATEMENT:

I understand that in the unlikely event of injury to my child resulting directly from child's participation in this study if such injury were reported promptly, the appropriate treatment would be available to the child. But, no further compensation would be provided by the hospital.

I understand that by my agreements to participate in this study and not waiving any of my legal rights.

I have explained to_____the purpose of the research, the procedures required and the possible risks to the best of my ability.

DR. PIDIKITI LAVANYA Date16/12/2022

(Investigator)

PARENTS / GUARDIAN CONSENT STATEMENT:

We confirm that Dr. Pidikiti Lavanya is doing a study on **OUTCOME OF NEONATAL RESUSCITATION DONE AT BIRTH IN NEWBORNS, CORRELATION WITH MATERNAL FACTORS AND MEASUREMENT OF OXIDATIVE STRESS LEVEL AND TO ASSESS THE LEVEL OF OXIDATIVE STRESS AND ITS CORRELATION TO THE OUTCOME** In NICU In Shri B. M. Patil Medical College Hospital, Vijayapura, Karnataka. Dr. Pidikiti Lavanya has explained to us the purpose of research and the study procedure. We are willing to allow our child to get treated in Shri B.M. Patil Medical College Hospital, Vijayapura. We have been explained about the study in our own understandable language , benefits and possible discomforts in detail in our native language and we understand the same. We are aware that child will get the best treatment, and no compensation like financial benefits will be given if our child's condition deteriorates and any untoward complication happens, and we will not sue anyone regarding this. Therefore we agree to give our full consent for the child's participation as a subject in this research project.

(Parents/Guardian)
Date:

(Witness to signature)
Date:

ANNEXURE – III

PROFORMA

NAME :

AGE:

SEX:

IP NO. :

MOBILE NO:

ADDRESS:

DATE OF BIRTH:

DOA TO NICU:

DATE OF DISCHARGE/DEATH:

GESTATIONAL AGE:

GRBS AT TIME OF ADMISSION:

SpO_2 AT TIME OF ADMISSION :

MATERNAL HISTORY :

AGE:

OBSTETRIC SCORE :

CONSANGUINITY :

LMP : EDD:

MOTHER'S BLOOD GROUP :

RISK FACTORS :

Abortions/stillbirths- Thyroid disease -

Anemia- mild/mod/severe

Tuberculosis-

PIH - BP - consciousness- Convulsions-

Asthma

PROM –

Prematurity -

PreviousC-section - Epilepsy-

UTI- heart diseases

DATE & TIME OF DELIVERY:

MODE OF DELIVERY:

BIRTH WEIGHT:

APGAR SCORE AT BIRTH:

APGAR SCORE AFTER 5 MINUTES:

SARNAT SCORING TOOL:

Normal /Mild	Moderate HIE	Severe HIE
1.Level of Consciousness Code 1: arouses to wakefulness, responds to external stim, or appears hyperalert or inconsolable/irritable	Code 2: Lethargic: delayed but complete response to external stimuli (start with mild stimuli first then proceed to more noxious stimuli)	Code 3: stupor/coma: infant is not arousable and is non-responsive to external stimuli; may have a delayed but incomplete response to stimuli
2. Spontaneous Activity Code 1: infant is active	2 = Decreased activity Code 2: activity is decreased	Code 3: no activity
3. Posture Code 1: if the infant is moving around and does not maintain one posture, should have flexion of lower extremity at hip and/or	2 = Distal flexion, complete extension Code 2 if strong distal flexion, complete extension or "frog-legged" position (complete abduction)	Code 3 if decerebrate with or without stimulation
4. Tone Code 1 if there is normal resistance	2a = Hypotonia (focal or general) 2b = Hypertonia Code 2: 2a if hypotonic or floppy either focal or generalized 2b if increased tone noted	Code 3: 3a if flaccid (like a rag doll) 3b if rigid (stiffness or inflexibility)
5. Primitive Reflexes * Suck Code 1 if the infant vigorously sucks the examiners finger or the ET tube	Code 2 if suck is weak or if infant has a bite Code 2 if incomplete	Code 3 = Suck is Absent Code 3 = Absent

*Moro Code 1 if, with stimulus, there is extension of limbs, opening of hands extension with abduction of UE		Code 3 if absent
6. Autonomic System		
*Heart rate Code 1 if >100 per min or consistent tachycardia	Code 2 if bradycardia (< 100/min) with only occasional increases to >120/min	Code 3 if heart rate is not constant and varies widely between 120
*Respiration Code 1 if breathing spontaneously or if periodic breathing without desaturation	Code 2 if periodic breathing associated with desaturations (SpO2 < 80%) ± supplemental O2	Code 3 if apnea or requiring ventilator support: •3a if spontaneous breaths above the ventilator •3b if no spontaneous breaths above the vent
*Pupils Code 1 if normal in size and reactive to light	Code 2 if constricted and reacting to light Code 3 if skew deviation of eyes, pupils are	Code 3 if skew deviation of eyes, pupils are dilated or non-reactive to light •If pupils asymmetric, assign 3

LEVEL OF RESUSCITATION:

Physical stimulation/ free flow oxygen/ bag and mask /intubation / CPR /medications

DURATION OF NICU STAY:

MALONDIALDEHYDE LEVEL IN CORD BLOOD:

INVESTIGATIONS

Hematological. -

Hb- PCV- Tc - N/L/E/M- Plt count- CRP-

ABG-

OTHERS:

DIAGNOSIS:

DocuSign Envelope ID: 1EE0B2C3-35A0-454D-92B4-AB980167C9DC

AT BIRTH)