"CLINICAL AND HEMATOLOGICAL MARKERS TO PREDICT SHORT TERM OUTCOME IN BIRTH ASPHYXIA."

BY
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I hereby declare that this dissertation/thesis entitled**"CLINICAL AND HEMATOLOGICAL MARKERS TO PREDICT SHORT TERM OUTCOME IN BIRTH ASPHYXIA"** is a bonafide and genuine research work carried out by me under the guidance of **Dr. S V PATTL, M.D** Professor & Head, Department of Pediatrics, Shri B.M. Patil Medical College Hospital and Research Centre, Vijayapur.

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Dr SHARATH KEERTHY.R

LIST OF ABBREVIATIONS USED

AAP	American Academy of Pediatrics
ABO	Blood Group A B And O
ACOG	American College of Obstetrician and Gynecology
ADH	Anti-Diuretic Hormone
AMPAR	A-Amino-3-Hydroxy-5-Methyl-4-Isoxazolepropionic Acid Receptor
ATP	Adenine Tri Phosphate
BAX	BCL-2 Associated X Protein
BAD	BCL-2 Associated Death Promoter
CBC	Complete Blood Count
CPAP	Continuous Positive Airway Pressure
CSF	Cerebral Spinal Fluid
DA	Dopamine
DAMA	Discharge Against Medical Advice
DAMP	Damage Associated Molecular Pattern
EAAT	Excitatory Amino Acid Transporters
ERCC	Excision Repair Cross Complementary Rodent Repair Group
HI	Hypoxic Ischemia
HIE	Hypoxic Ischemic Encephalopathy
ICD	International Classification of Diseases
IL	Interleukin
IUGR	Intra Uterine Growth Retardation
MMP	Matrix Mettalo Proteinase
MR microscopy	Magnetic Resonance Microscopy
MRI	Magnetic Resonance Imaging
NALS	Neonatal Advanced Life Support
NDP	Nicotinamide Adenine Dinucleotide Phosphate
NFKB	Nuclear Factor Kappa Light Chain Enhance of Activated B Calls
NMDA	N Methyl D Aspartic Acid

NNF	National Neonatal Forum
NNPP	National Neonatal Perinatal Database
NICU	Neonatal Intensive Care Unit
PA	Perinatal Asphyxia
PARP	Poly ADP Ribose Polymerase
pH	pouvoirHydrogène
PNC	Post Natal Care
RBC	Red Blood Cell
Rh	Rhesus
RNA	Ribo Nucleic Acid
SD	Standard Deviation
SES	Socio Economic Scale
SPSS	Statistical Package For The Social Sciences
TH	Tyrosine Hydroxylase
TLC	Total Leukocyte Count
TLR	Tall Like Receptor
TNF	Tumor Necrosis Factor
TORCH	Toxoplasma Rubella Cytomegalovirus Herpes Virus
WBC	White Blood Cell
WHO	World Health Organization

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ABSTRACT

BACKGROUND

Birth Asphyxia is a sequence of events defined as failure to initiate and sustain breathing at birth and associated with reduction in the arterial oxygen tension, accumulation of carbon di oxide and fall in blood pH. Acidosis occurs due to anaerobic utilization of glucose, production of lactic acid and accumulation of Co_2. These biochemical changes cause constriction of relatively muscular pulmonary arterioles and raise the pulmonary arterial pressure. This results in reduced filling of left heart and right to left shunts. The hypoxic event induces a compensatory response in the form of exaggerated erythropoiesis, resulting in the release of immature red blood cells (NRBC) and Reticulocytes into the fetal circulation. The levels of NRBC and Reticulocytes may be correlated with the presence of perinatal asphyxia. These Physio-chemical changes perpetuate asphyxia, unless corrected by therapy.

OBJECTIVES OF THE STUDY

1. Nucleated RBC count/100 WBC's, Absolute Nucleated RBC Count and Reticulocytes Count in Cord Blood and/or neonatal venous blood (Hematological Factors).
2. Correlation of Risk Factors and Hematological factors with Short term Outcome.
3. Short term outcome of Asphyxiated Babies.

METHODOLOGY

A prospective observational study involving 250 term babies; 125 cases satisfying selection criteria in NICU and PNC Ward and 125 controls are included over one and

half year. After informed consent, nRBC/WBC and Reticulocyte count were performed on:

- Cord Blood in inborn babies
- Peripheral Venous Blood in out born Babies presenting <24 hours of birth.

Neonates were followed up till discharge or death. Morbidities and duration of stay were noted.

RESULTS

- Out of 125 cases Reticulocyte count range was **0.34% to 12%** and mean was **6.36%** where as in controls Reticulocyte count range was **1.4 to 8%** and mean was **4.09%** With **p-value <0.001.**
- nRBC count range was **0 to 90** and mean was **9.5** where as in controls nRBC count range was **0 to 23** and mean was **2.6** with **p-value 0.002** at 5% level of significance ($p<0.05$).
- 34 cases (27%) and 8controls (6.4%) had high Reticulocyte count. 48 cases (38.4%) and 1 control (0.8%) had high nRBC count.
- 20 cases (38%) were neurologically abnormal.
- 16 cases (69.5%) with high retic count had duration of stay >5days and 13cases (81.2%) with high nRBC had duration of stay >5 days.
- Among 20 neurologically abnormal cases 9(47%) had high reticulocyte count and 14 had high nRBC count.
- All these above parameters are compared and correlated with 125 normal new-born (controls) from post-natal ward.

CONCLUSION

Mean NRBC count and Retic counts are significant in Birth Asphyxia cases when compared with normal neonates. They have significant positive correlation with severity of hypoxic ischemic encephalopathy, neurological impairment following birth asphyxia, time taken for recovery of neurological impairment following birth asphyxia, duration of NICU stay. It can be concluded that Nucleated red blood cell count and Reticulocyte count counts are simple markers for assessment of severity and early outcomes of perinatal asphyxia and duration of stay in NICU.

KEYWORDS

Perinatal Asphyxia, Nucleated RBC, Reticulocyte Count, Hypoxic Ischemic Encephalopathy.

INTRODUCTION

INTRODUCTION

*"**Our population consists of about one third of Children and all of our future"***

Perinatal asphyxia is a serious problem globally and is one of the common causes of neonatal mortality. According to world health organization (WHO), birth Asphyxia is failure to initiate and sustain breathing immediately after birth. It is the third major cause of neonatal death after infections (1). About 30% of out born Babies and 5% of inborn babies admitted in our NICU are due Birth Asphyxia. New born babies may not breathe at birth due to many causes originating at different periods of the pregnancy. Birth asphyxia may primarily be due to complications occurring during the ante partum (50%) intrapartum (40%), and postpartum (10%) periods. Therefore, to reduce the incidence of birth asphyxia, interventions must be directed towards addressing the conditions that occur during each period when birth asphyxia occurs.

DEFINITIONS:

1) "American Academy of Pediatrics (AAP) and American College of Obstetrics and Gynaecology (ACOG) define perinatal asphyxia when all the following criterias are met.
    - Profound metabolic or mixed acidemia (pH < 7.00) in an umbilical arterial blood sample.
    - Apgar score of 0-3 > 5 min. after birth.
    - Neonatal encephalopathy (e.g., Seizures, coma, hypotonia).
    - Multiple organ involvement (Kidney, lungs, liver, heart, intestines)"(2).

2) **"The National Neonatal Forum(NNF)** has recommended three definitions. The first and second are for hospitals, while the third is for use in the community level.

Definition 1:

- Moderate birth asphyxia: Slow gasping breathing at one min of age. Heart or Cord pulsation rate should be recordable.
- Severe birth asphyxia: No breathing at one min of age. Specify whether the Heart beat or Cord pulsation is present or not.

Definition 2:

- Moderate birth asphyxia: Apgar score 4-5 at one min of age.
- Severe birth asphyxia: Apgar score 3 or less at one min of age.

Definition 3:

- A new born is said to have suffered from birth asphyxia if she/he after birth has absent or weak cry or had absent or slow gasping respiration or any baby who need resuscitation measures beyond initial steps as per NALS (neonatal advanced life support). Thus, if a neonate requires oxygen, bag and mask ventilation, drugs or intubation for initiation of respiration it will constitute birth asphyxia".

3) "The National Neonatal and Perinatal Database (NNPD) defines perinatal asphyxia as Apgar score of <7 at one minute of life. NNPD also defines moderate asphyxia as slow gasping breathing or an Apgar score of 4-6 and severe asphyxia as no breathing or an Apgar score of 0-3 at one minute of life". (3)

The International Classification of Diseases (ICD) is the standard diagnostic tool for epidemiology, health management and clinical purposes. It is used to classify diseases and other health problems. ICD-10 was endorsed by the Forty-Third World Health

Assembly in May 1990 and came into use in WHO Member States as from 1994. Perinatal asphyxia is specified in chapter XVI under Respiratory and cardiovascular disorders specific to perinatal period (code P20 to P29).

STAGING SYSTEM

The staging system proposed by Sarnat and Sarnat in 1976 is often useful in classifying the degree of encephalopathy. Mild (stage I), moderate (stage II), or severe (stage III) HIE is commonly diagnosed using physical examination, which evaluates the level of consciousness, neuromuscular control, tendon and complex reflexes, pupils, heart rate, bronchial and salivary secretions, gastrointestinal motility, presence or absence of myoclonus or seizures, electroencephalography findings, and autonomic function.(41)

STAGE 1(MILD)	STAGE 2(MODERATE)	STAGE 3(SEVERE)
No seizures	Clinical seizures	Persistent seizures
Mild alterations in tone	Marked abnormalities of tone	Severe hypotonia
Suck intact	Weak suck	Absent suck
Exaggerated Moro	Moro incomplete	Moro absent
Pupils react normally	Pupils constricted	Deviated, dilated, or nonreactive
Hyperalert	Reduced responsiveness to sound, light, touch	Unresponsive
Jittery, tremor on handling	Reduced activity Distal flexion, proximal extension	No activity Extended, Impaired breathing

Table no 1 "Modified Sarnat HB, Sarnat MS. Neonatal encephalopathy following fetal distress. A clinical and electroencephalographic study" (41)

EPIDEMIOLOGY

Each year four million new born suffer from birth asphyxia all over the world. Of these one million die; and an equal number develop serious sequelae. Perinatal asphyxia ranks as the 2nd most important cause of neonatal death after infections accounting for about 23% mortality worldwide (1), Out of the 1.2million neonatal deaths every year in India about 300,000 to 350,000 neonates die due to perinatal asphyxia mostly within first three days of life (3).

Most of the diagnostic and prognostic parameters which used are available in a very few selected tertiary care hospitals which are expensive and require sophisticated equipment thus rendering them unreachable for most of the population. This problem is further compounded in country like India where there is a wide gap between the need and accessibility of health services.

The preventive aspects of neonatal asphyxia are very important. Intensive antenatal care to detect risk factors and adequate interventions or referral are vital aspects of prevention. Intra-natal assessment of fetal hypoxia and management of fetal distress should be done promptly. Special attention should be paid for avoidance of preterm delivery, care of preterm and low birth weight infant to prevent birth asphyxia. Hospital personnel in all levels of care should work to prevent this life- threatening condition.

Even though birth asphyxia can be predicted for few conditions such as fetal distress and preterm child birth, most cases of birth asphyxia cannot be predicted. Therefore, every newborn should be considered as risk of asphyxia. Any infant can have neonatal asphyxia without warning signs during labour. Therefore, all the medical

attendants must be competent in newborn resuscitation and must have the necessary equipment ready for the resuscitation of the newborn baby.

Considering the magnitude of problems, it is essential to find out risk factors clinically as well as hematological (which can act as marker) for the birth asphyxia in the local population so that a step towards prevention can be taken. The Correlation with these markers will help to predict and prevent the adverse outcome to some extent. Short term outcomes identified in this study will eventually determine long term outcome in these babies.

This study will highlight the risk factors in local population attending our hospital, so that an effort can be made to manage those for better outcome. Present study also tests the usefulness of nucleated RBCs and Reticulocytes in cord and neonatal blood as a marker of short term outcome and Neurological status at discharge for long term outcome.

OBJECTIVES

OBJECTIVES

To Study neonates with Birth Asphyxia in terms of

1. Nucleated RBC count/100 WBC's and Reticulocytes Count in Cord Blood and/or neonatal venous blood (Hematological markers).
2. Correlation of Risk Factors and Hematological markers with Short term Outcome.
3. Short term outcome of Asphyxiated Babies.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

HISTORICAL PREVIEW

Dr. Eastman of Hopkins called asphyxia "an infelicity of etymology" since the Greek derivation of asphyxia meant "without pulse."(5)

“Perinatal asphyxia is characterized due to impairment in exchange of oxygen and carbon dioxide (respiratory gases) resulting in hypoxemia and hypercapnia, accompanied and metabolic acidosis”(18).

In 1924, Lippman reported “NRBC in the blood of 41 of 42 new-born on the first day of life (6). These cells constituted about 500 NRBC/mm3 or 0.1% of the new-born circulating red blood cells”. The first studies to show relationship between fetal hypoxia and increase in NRBC were performed in 1930’s and 1940’s (6)(7)(8). In these early studies fetal hypoxia and other abnormal conditions like meconium aspiration syndrome and fetal anaemia were associated with increase in number of NRBC in the histopathology sections of placenta (6)(7)(8)(10).

In the first half of Pregnancy, nucleated red blood cells are present in placental blood vessels but are usually absent or in less numbers in later half of pregnancy or at term. Hence finding of many nucleated red blood cells in later pregnancy is non-specific and it may indicate acute or chronic fetal hypoxia, maternal diabetes, congenital TORCH or fetal anaemia. Fox found that “Acute asphyxia was the most common of these causes.” (10).

The reticulocyte is a young red cell. It is produced in the bone marrow where it normally has a life of 2.8 days (28). The reticulocyte count is an index of effective erythropoiesis, that is, it reflects the number of red cells being produced in the marrow

and entering the peripheral blood. It can also act as marker in hypoxia as erythropoiesis is triggered in chronic hypoxic states. There are no studies evaluating the prevalence of or factors contributing to reticulocytes as predictor for outcome of birth asphyxia.

PROGNOSIS AND SEQUELAE OF ASPHYXIA

Neuro developmental outcome studies after Hypoxic Ischemic Encephalopathy usually provide restricted information concerning children, pooling a good vary of outcome severities. The stress in neonatology and paediatrics is on non-invasive diagnosis approaches for predictive diagnostics. Many strategies for predicting outcomes in infants with Hypoxic Ischemic Encephalopathy are used in the clinical setting including: neonatal clinical examination and clinical course, monitoring general movements (71)(72), early electrophysiology testing, cranial ultrasound imaging, Doppler blood flow velocity measurements, magnetic resonance imaging (MRI) and MR microscopy. The neonatal brain MRI provides detailed information about lesion patterns in Hypoxic Ischemic Encephalopathy allowing for earlier and more accurate prediction of long-term outcome. a potential serum biomarker for predicting individual predispositions to pathologies or progression of complications induced by asphyxia has to be studied for easier and economical prediction for severity and outcomes of Perinatal asphyxia.

PATHOPHYSIOLOGY

According to Volpe, “Hypoxemia is outlined as the Diminished amount of oxygen in the blood supply, where as cerebral ischemia is outlined as the “Diminished amount of blood perfusing the brain”. Cerebral ischemia is the more important of the two forms of

oxygen deprivation as a result of it, leads to glucose deprivation. The terms hypoxia-ischemia and asphyxia are typically used interchangeably, however they're not equivalent, from a pathophysiological viewpoint. Hypoxia-ischemia or pure ischemia are seldom observed in the newborn, whereas some combination of hypoxia, ischemia and hypercapnia are more common (19). After perinatal insult resulting in hypoxic-ischemia, totally different sequences of pathologic events occur, resulting in brain injury. In neonates, the phases of primary and secondary energy failure are recognized,on supported characteristics of the cerebral energy level (20). Within the section of primary energy failure, reductions in cerebral blood flow, in O_2/substrates and in high-energy phosphorylated compounds (ATP and phosphocreatine) are observed; What is more, tissue acidosis is outstanding. This section represents a necessary requirement for all later pathologic events. Primary energy failure is related to a complex series of acute intracellular derangements, as well as loss of membrane ionic physiological state, defective osmo regulation, release/blocked re-uptake of excitatory amino acids, and inhibition of the synthesis of proteins (21). Overstimulation of neurotransmitter receptors, related to loss of ionic physiological state, mediate an elevation in intracellular calcium and osmotic dysregulation. The increase in intracellular calcium triggers many destructive pathways by activating proteases, lipases and endonucleases (22).

The fall in high-energy phosphorylated compounds and intracellular pH later on is reversed, and utilisation of neuro transmitters is promoted, if the resolution of hypoxia-ischemia happens at a particular interval of time; the period of this interval is laid low with numerous factors together with maturation, substrate convenience, body temperature and concurrent pathological conditions. If the injury is sufficiently severe, a secondary

energy failure happens within hours to days after the primary insult. Secondary energy failure is characterised by declines in Creatine phosphate and Adenosine triphosphate without brain acidosis, totally different from primary energy failure (20).

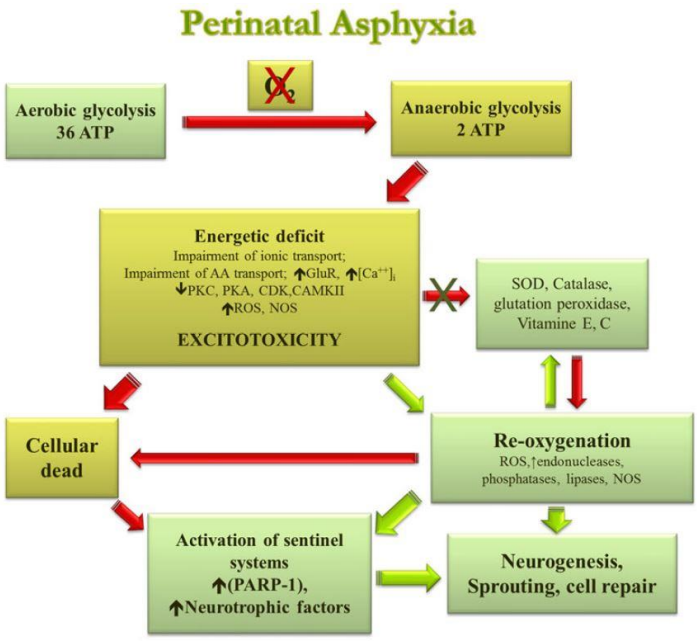


Figure no 1: Perinatal Asphyxia and Cell Death- Pathophysiology

PERINATAL ASPHYXIA AND CELL DEATH

The mechanisms of neuronic cell death after PA includes necrosis, apoptosis, autophagia and hybrid cell deaths and/or a time of neuronic phenotypes, relying primarily on the severity of the insult and the maturational state of the cell (73-77). An initial decrease in high-energy phosphates ends up in impairment of the ATP-dependen tNa+-K+ pump, then when the severe insult causes associate degree of acute inflow of Na+, Cl−, and H_2O with resultant cell swelling, cell lysis, and so early cell death by necrosis. Conversely, a less severe insul tcauses membrane depolarization followed by a cascade of excite toxicity and oxidative stress, resulting in delayed cell death, primarily

caspase-mediated cell death.Thus ,necrosis are often observed within minutes, whereas caspase-mediated cell death takes longer time to develop (78). caspase-mediated cell death is triggered by the activation of endogenous proteases caspases, leading to cyto skeletal disruption, cell shrinkage, and membrane blebbing. The nucleus undergoes chromatin condensation and nuclear de oxy ribonucleic acid degradation ensuing from endonuclease activation (79). Since caspase-mediated cell death needs energy, a determinant issue of when cells die is probably the ability of mitochondria to produce adequate energy. Another determinant of classic caspase-mediated cell death is that the loss of neuronic connections, which may continue days to weeks after injury, as a result of groups of cells appear to commit to die(74). Apoptosis is the more prevalent type of delayed cell death with in the perinatal brain, and each caspase-dependent and caspase-independent mechanisms of apoptotic cell death are recognised (73,80-83). Thus, multiple cell death mediators are activated by neonatal Hypoxic Ischemic injury, together with numerous members of the Bcl-2, Bcl-2-associated X protein (BAX), Bcl-2-associated death promoter(BAD)(80,84,85) death receptor (86), and caspases (87,88) protein families, correlating with accumulated caspase-mediated cell death within the brain (89,90). After neonatal insult, markers of caspase-mediated cell death (cleaved caspase-3) and necrosis (calpain-dependent fodrin breakdown product) are often expressed by a similar broken neuron(91),suggesting that the “continuum” might be explained by a failure of some dying cells to complete caspase-mediated cell death, due to a absence of energy and mitochondrial dysfunction (74,83,92). HI conjointly will increase markers for autophagosomal(microtubule-associated protein 1 light chain 311) and lysosomal activities (cathepsin D, acid phosphatase, and β-Ncetylhexosaminidase) in

cortical and hippocampal CA3-broken neurons, suggesting an activation of autophagic flux that may be related to the caspase-mediated cell death ascertained in delayed neuronic death after severe HI (76,93).Accumulated information of the factors that confirm when or however cells die after HI is very important since it would be do able to salvage tissue using drugs, growth factors, or interventions that influence brain activity and restore the damaged neurocircuitry. Perinatal asphyxia and neuro transmission systems Glutamatergic system the depletion of energy reserves that accompanies prolonged hypoxia ends up in neuronic depolarisation and the release of excitatory amino acids into the extra cellular space (73,94-97), in concentration that exceed each the glial reuptake capability that's more compromised by energy failure (98) and re-uptake into the conjunction nerve terminal (99). Thus, glutamate and aspartate accumulate to excite toxic levels (100-104). Glutamate activates ionotropic NMDA, AMPA/KA and metabotropic receptors .AMPA/KA receptor activation increases sodium electrical phenomenon,depolarising the membrane and activating voltage dependent calcium channels together with the NMDA receptor channel. Metabo tropic receptors mGluR1-mGluR5, through second messengers, mobilise calcium from intra cellular reservoirs to the cytosolic compartment, activating proteases, lipases and endonucleases, that successfully initiate a method of celld eath (100,104-106). In fact, a transient increase in excitant amino acid levels has been found in many experimental models of Hypoxic Ischemia within the cerebro spinal fluid of neonate (96,127,107,108). The importance of NMDA-mediated injury within the immature brain is expounded to the actual fact that NMDA receptors are functionally up regulated within the perinatal period due to their role in activity-dependent neuronal plasticity (103).Immature NMDA channels features a

higher likelihood of aperture and electrical phenomenon than adult channels, and also the voltage-dependent magnesium block that's unremarkably present in adult channels at resting membrane potentials, is lot of easily relieved in the perinatal period (93,109).Thus, increased expression and phosphorylation of NR1 subunits of NMDA receptors are discovered within the corpus striatum once PA. This alteration is related with increased excitability and neuro degeneration throughout the neonatal period (110,111). Moreover, a deficiency within the GluR2 subunit of AMPARs throughout development has been related with increased susceptibility to Hypoxic Ischemia at the regional and cellular levels (112,113). Recent studies more recommend cross talk between inflammation and excitotoxic neuronal damage. It's been shown that the pro-inflammatory protein TNF-α is one among the fore most potent regulators of AMPAR trafficking to and from the plasma membrane, which it will rapidly increase the proportion of Ca2+-permeable AMPAR at the surface together in combination with increased extra cellular glutamate levels, this enhances excitotoxic cell death (127,114).The pharmacological blockade of glutamate receptors markedly protects against brain injury evoked by severe hypoxia (115-118), reinforcing the concept that glutamatergic receptors throughout the perinatal period are most inclined over-activation, promoting the excitet oxicity found after hypoxic ischaemic insults.Astrocytes conjointly play a crucial role in preventing neurotoxicity by glutamate uptake (119-123) and are plagued by the energy deficit evoked by PA as represented earlier. Indeed, a decrease in glutamate uptake has been determined within the hippocampus of rat pups subjected to fifteen min of PA (124) and an identical result has been observed within the cortex, basal ganglia and thalamus of piglets (125).Reduced glutamate uptake is correlated with a

down regulationof astrocytic excitatory amino acid transporters(EAAT-1 and EAAT-2)(126) after Hypoxic Ischemia, reinforcing the concept that energy deficits conjointly promote a severe disruption of astrocytic cell function.

DOPAMINERGIC AND NITRIDERGIC SYSTEM

Mesencephalic dopamine (DA) neurons are essential for the management of motor and psychological feature behaviour, and are related to multiple psychiatric and neurodegenerative disorders (128). In recent years, increasing proof shows that the mono amine neurotransmitters, mainly Dopamine, could aggravate harm to the brain induced by Hypoxic Ischemia. The striatum, a section richly innervated by the nigrostriatal dopaminergic pathway, is particularly prone to asphyctic neura lharm (129). Levels of Dopamine as well as its metabolites could stay elevated even after normoxia is stabilised (130), due to an impaired Dopamine uptake mechanism (131,132). It has been suggested that during Hypoxic Ischemia, the rise in extracellular Dopamin elevels may end up in alterations within the sensitivity of neurons to the excitative amino acids (133,134). Moreover,glutamate and aspartatel evels are increased, primarily in mesencephalic tissues (135). A planned mechanism for the toxin impact of Dopamine is through a rise in the production of free radicals through out the re-oxygenation period (136,137). This is often in agreement with proof showing that neural injury occurring throughout re-oxygenation after anasphyctic insult is partly due to oxygen free radical mediated oxidative events (138-140). Perinatal Asphyxia additionally induces change within the expression and pharmacological parameters of dopaminergic receptors within the meso-telencephalic Dopaminesystems (96). Additionally, asphyxia induces an increase of

tyrosine hydroxylase (TH) messenger RNA within the projection fields, corpus striatum and limbic regions, at first week. Perinatal Asphyxia did not appear to exert any effect on D1R messenger RNA levels. These changes could have an effect on D2R and D1R expression differently throughout development, contribute to long-run imbalances in neurocircuitry (141).The postnatal establishment of Dopamine neural connectivity are of disturbed by metabolic insults occurring at birth. Indeed, it has been shown that Perinatal Asphyxia, alters the establishment of Dopamine neurocircuitries, with long-run consequences (142).

PERINATAL ASPHYXIA AND NEUROINFLAMMATION

Recently, the interconnection between the immune and neuronal systems has been a spotlight of many studies, particularly within the context of pathologic processes, within which sustained or excessive inflammation has been related to neurotoxicityand various neuropathology (158-161).One major hallmark of neuro-inflammation is that the activation of neuroglia, which are resident parenchymal cells of the brain, derived from a similar myeloid lineage as macrophages and dendritic cells (162). If brain injury happens, neuroglia activates, dynamic pattern of secreted molecules and activating de novo synthesis of inflammation-related molecules (163). Microglial activation has useful effects for the removal of cell junk, that attenuates inflammatory responses and promotes the remodelling of the affected space. However, over-activation of neuroglia will exacerbate neuronal death, as a result of inflammatory molecules contribute to a detrimental setting,inflicting secondary harm(164). Hence, the balance between a properly modulated or exacerbated im mune response is prime for biological

homeostasis. Following Hypoxic Ischemia, native inflammation is created by activated neuroglia (165), in all probability due to necrotic cell death, manufacturing a **Damage-associated molecular pattern** (DAMPs). **Toll-like receptors** (TLRs) are expressed by neuroglial cells (159),sensing the DAMPs (166) and causing the activation of the key transcription factor related to inflammatory response, i.e. NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells). Following asphyctic injury, NF-κB is quickly activated in neurons and glial cells (167,168). Indeed, it has been shown that NF-κB p65 is up-regulated within the rat brain 10 min post-Perinatal Asphyxia(169). A rise within the transcriptional function of NF-κB due to neuroglia activation ends up in to the induction of many genes related to the innate immune reaction, together with proinflammatory cytokines such as: **Tumoral necrosis factor-α** (TNF-α), **Interleukin-1 beta** (IL-1β) ,**Interleukin-6** (IL-6), **Interleukin-10** (IL-10), **Interferon gamma** (INF-γ), and proteases such as **Matrix Metallo proteinases three** and **nine** (MMP-3 and MMP-9) (170-173) .Inhumans, a relationship has been established between pro-inflammatory cytokine serum level and outcome for infants with Perinatal Asphyxia. "Infants who die or develop cerebral palsy had high plasma levels of pro-inflammatory cytokines as compared to infants with normal outcomes" (174). Inagreement, blood levels of IL-1β, IL-6 and TNF-α measure correlate with cerebral spinal fluid (CSF) levels of IL-1β in infants with HIE during the first twenty-four hours of life (175). Thus, cell damage during Perinatal Asphyxia is related to neuroglia mediated inflammation (176) and inflammatory markers is also hepful in predictive diagnostics for Perinatal Asphyxia evokedbrain damage and clinical outcomes. Perinatal asphyxia and sentinel proteins negatively affects the integrity of the genome, triggering the activation of sentinel

proteins that maintain genome integrity, such as poly(ADP-ribose) polymerases (PARPs)(177), X-Ray Cross Complementing Factor 1 (XRCC1),DNA ligase IIIα(178), DNA polymerase β(179,180),Excision Repair Cross-Complementing Rodent Repair Group 2 (ERCC2)(169,181,182) and DNA-dependent protein kinases (183).PARP-1, a member ofthe nuclear chromatin-associated PARPs proteins. PARP-1 catalyses the formation of poly(ADP-ribose) polymers (pADPr) from nicotinamide adenine dinucleotide (NAD+), releasing nicotinamide as a product (184,185). pADPris then transferred to glutamic acid or aspartic residues of acceptor proteins,modifying them post-translationally (185,186).Once PARP-1 is activated, intracellular levels of Pad Princrease about 10 to 500 times (185). Activated PARP-1 acts as a transcription regulator, unravelling the super structure of chromatin granule and regulation of the transcriptional activity of varied genes,together with nitric oxide synthase, chemokines and integrins.Thus, PARP-1 is concerned within the regulation of varied processes, together with Deoxyribonucleic acid replication, repair, transcription, mitosis, proteins degradation and inflammation (185).Despite the useful effects of PARP-1 activation for necessarycellular functions, increased pADPr formation may be detrimental, resulting in variedtypes of cell death (188). Normally, in mild Deoxyribonucleic acid damage, PARP facilitates Deoxyribonucleic acid repair by interacting with DNA repair enzymes such as De oxyribo nucleic acid polymerase, XRCC1 and DNA-dependent protein inase, permitting cells to survive. once the De oxy ribonucleic acid damage is irreparable, caspase-dependent cell death, mediated by caspase 3 and caspase 7, degrades PARP-1 into 2 fragments of 89 and 24 kDa (189). Therefore, the cell is eliminated by programmed cell death. It has additionally been reported that the build up of pADPr

promotes the release of AIF (Apoptosis-inducing factor) from the mitochondria, resulting in cell death through caspase-independent apoptosis (185). However, once Deoxyribonucleic acid damageis severe, PARP-1 is over activated, depleting intracellular NAD+ levels, and consequently ATP (190). This energy-compromised state inhibits several cellular processes, together with apoptosis, and promotes necrosis (191). Severe DNA damage is sometimes triggered by a massive degree of oxidative stress triggered by reactive oxygen species such as peroxy nitrite, hydroxyl and superoxide free radicals. Thus, the effect of PARP-1 activity depends greatly on the intensity of DNA damage. Asphyctic injury is characterized by low energy accessibility, owing to an absence of oxygen. During this context, PARP-1 over activation is particularly important for cell survival. Several asphyctic models report the importance of energy depletion during this clinical condition (192-194) and note that PARP-1 inhibitors will avoid excessive energy decreases (195-197). Systematically, restoring NAD+ will stop changes evoked by PARP-1 over activation (177).

MULTISYSTEM ORGAN INVOLVEMENT

The consequences of hypoxic-ischemic insult sometimes be extended to other organ systems in additionally to the brain. During a minority of cases (< 15%), the brain is that only organ that exhibits disfunction post Perinatal asphyxia. In most cases, systemic hypoxia-ischemia ends up in multiorgan dysfunction.

Respiratory System

For the entire Duration of intrauterine life the foetal lungs are crammed with fluid and they don't serve any ventilatory use,as the placenta provides oxygen to foetus. The blood flow through the lungs is markedly diminished because of constricted arterioles & right to left shunts through the Ductus Arterious throughout foetal life. Throughout vaginal delivery, one-third of foetal lung fluid is eliminated as the chest is squeezed and lung fluid comes out through nostril and mouth. The primary few breaths of many of the new born babies are extremely effective to inflate the alveoli and replace the lung fluid with air. Infants who are apnoeic at birth and those having weak respiratory efforts cannot succeed this function."The lungs of asphyxiated neonates can be injured by asphyxic hypoxia, as a result of inhalation of meconium, secondary to cardiac disfunction, or compromised because of pulmonary high blood pressure"(24). Consequently, gas exchange is impaired and mechanical assisted ventilation may be needed.

Cardio Vascular System

Hypoxic-ischemic injury causes a directed damage to the heart muscle along with the poor consequences of counter vailing mechanisms to keep up cerebral perfusion, results in a recognizable clinical and laboratory picture (25) (26). Cardiac Muscular ischemia compromises cardiac conduction & contractile efficiency, usually requiring an inotropic support to keep up adequate circulation. Functional and conduction abnormalities could also be detected using Diagnostic Procedures like, Cardiac echo and ECG, whereas cardiac muscle damage is mirrored by the increase of cardiac enzymes.

Renal System

Kidney injury represents the most effective systemic marker of cerebral injury. Oligo-anuria following hypoxic-ischemic injury is common, regularly associated with haematuria, and results from renal tubular damage. Concentrations of Serum Creat and blood Urea increase steadily, achieving the peak within few days following the perinatal asphyxia injury. Fluid retention and hypo natremia could arise because of in appropriate secretion of ADH.

Hepato-Biliary System

Liver dysfunction may be manifested by increased hepato cellular enzymes, even though more extensive damage may develop.

Bone Marrow

The effects on the bone marrow include an increased release of nucleated red blood cells (NRBC) and thrombocytopenia. The NRBC count reaches a peak at 6-8 hours following brain injury and returns to normal by 36-72 hours. On the contrary, the thrombocytopenia occurs sometimes by 12 hours, and reaches the nadir at 2-3 days. Thrombocytopenia can be worse enough to determine or aggravate haemorrhage (risk of intracerebral bleeding).

Other Manifestations

Fluctuations of blood sugar concentration is also ascertained, with hypoglycaemia being most typical. Hypoglycaemia could lead to neurologic sequelae, significantly once it causes or accompanies seizures. On the contrary, hyperglycaemia might also result in, or aggravate, brain injury through a mechanism involving a hyperosmolar state (27).

CAUSES OF INCREASE IN NRBC

The precise mechanism(s) inflicting the speedy release of Nucleated red blood cell following acute asphyxia isn't known. The proposed mechanism for a rise in the Nucleated red blood cell is that the production of erythropoietin. The sole acknowledge stimulant for erythropoietin is tissue hypoxia, that has been well documented in human fetuses. Knowledge from studies on animals and adults suggests that erythropoietin will increase within one to four hours of hypoxia (29)(30)(31) Erythropoietin is a glycoprotein, that doesn't cross the placenta, therefore the levels in the fetus are fetal in origin. (31,32) Throughout the yolk sac stage, erythropoiesis is erythropoietin insensitive. Its not acknowledged whether or not insensitiveness is because of erythropoiesis being erythropoietin non dependent or erythropoiesis is maximally already stimulated by erythropoietin. (33,34,35) Once the yolk sac stage, fetal progenitor cells are very erythropoietin sensitive (36) In the normal fetus, erythropoietin levels are very low throughout second and third trimester and are not gestational age dependent.(31,36) It is probably that the increased number of circulating Nucleated red blood cell represents erythropoietin evoked release of normoblasts from their marrow stores. High titres of erythropoietin are shown to accelerate mitotic divisions of the normoblasts, increase

blood flow through the bone marrow and increase the porous structure of the bone marrow, permitting escape of comparatively giant and rigid normoblasts.(37,38,39,40) These every processes contribute to a shorter marrow transit time and speedy release of normoblasts into the blood stream after acute hypoxia. Although nucleated red blood cells (NRBC) are seldom found circulating in older children, they'reunremarkably seen within the blood of neonates. They're primarily created within the fetal bone marrow in response to erythropoietin and are stored within the marrow as precursors to reticulocytes and mature erythrocytes. Several acute and chronic stimuli will increase the number of circulating Nucleated red blood cells from either increased erythropoietic activity or a unforeseen release from the marrow storage pools. Prematurity, chronic hypoxia, maternal anaemia, maternal diabetes mellitus and acute perinatal stress are most common.

As of currently no correct studies are conducted where reticulocyte counts are enclosed in studies related to birth asphyxia. And this is a platform to see the association of reticulocyte counts as well as its variation according to severity of the asphyxia.

Differential Diagnosis Of Increased Nucleated Red Blood Cells and Reticulocyte counts In The Fetus And New-born

I. Physiological

- Labour and vaginal births
- Preterm new-borns
- Post-term new-borns

II. Increased erythropoiesis

- Chronic hypoxia
  - Growth restriction
  - Maternal pre-eclampsia
  - Maternal smoking
- Anaemia
  - Blood loss
  - Haemolysis—ABO or Rh isoimmunization, other
- Maternal diabetes
- Other
  - Leukaemia
  - Down's syndrome
  - TORCH infections*

III. Acute stress release

- Acute hypoxia
- Subacute hypoxia
- Chorioamnionitis

IV. Postnatal hypoxia

- Cyanotic heart disease
- Pulmonary failure

V. Idiopathic

- **TORCH – Toxoplasmosis, others, rubella, cytomegalovirus virus and herpes*

Chronic hypoxia

Tissue hypoxia leads to augmented levels of erythropoietin, that in turn results in stimulation of erythropoiesis & augmented numbers of circulating Nucleated Red blood cells. Increased umbilical cord levels of erythropoietin have been found in pregnancies complicated by intrauterine growth restriction, maternal hypertension, pre-eclampsia, maternal smoking, Rh isoimmunisation and maternal diabetes (42,43,44,45,46) As expected, every condition has been associated withincreased Nucleated Red blood cells in the neonate. IUGR is a common manifestation of chronic hypoxia. Studies have found Nucleated Red blood cells counts in growth restricted preterm and term infants to be about twice the values in normal controls. The counts tend to increase with worsening foetal arterial and venous Doppler flow measurements. Raised Nucleated Red blood cells have additionally been found in infants presumed to have experienced plausible chronic hypoxia due to maternal pre-eclampsia with or without growth restriction (47) Yeruchimovich et al compared non - growth restricted, term infants of smoking mothers with normal controls (51) The infants of smoking mothers had considerably augmented numbers of Nucleated Red blood cells, with a correlation statistics between the numbers of cigarettes smoked every day and also the Nucleated Red blood cells count. Even passive smoking of the mother has been related to slightly augmented neonatal Nucleated Red blood cell counts (49)These studies support the theory that mild, however prolonged, foetal hypoxia will induce erythropoiesis and augmented Nucleated Red blood cells.

Maternal diabetes

"In 1944, Miller et al 1st reported the presence of increased Nucleated Red blood cells and extramedullary erythropoiesis in infants of diabetic mothers". Additionally, "Greenand Mimouni reported that asphyxiated infants of diabetic mothers had 1800 ± 2300NRBC/mm3, non-asphyxiated infants of diabetic mothers had 1400 ± 3100NRBC/mm3, and normal control infants had 400 ± 1300NRBC/mm3" (50)

The values from the two diabetic clusters were considerably beyond than that of the control cluster, however weren't considerably completely different from each other. "Hanlon-Lundberg et al found14.6 ± 12.2 NRBC/100 WBCs in infants of diabetic mothers, compared with 8.3 ±10.1 NRBC/100 WBCs in control infants whose mothers weren't diabetic (51)" Infants of diabetic mothers who are large for gestational age have higher Nucleated Red blood cell counts than those who are of appropriate for age (52). The enhanced erythropoiesis is may be because both an increase in erythropoietin levels and a direct haematopoietic effect of hyper insulinemia (53)

Blood loss and haemolysis

Blood loss and haemolysis are potent stimulants of erythropoietin and increased NRBC. Haemolysis from any cause can result in an increase in circulating Nucleated Red blood cell (46)

Other chronic causes

Other less common causes of long standing erythropoiesis are Down's syndrome, TORCH (congenital toxoplasmosis, other, syphilis, rubella,cytomegalovirus) infections

and parvovirus have all been related to increased Nucleated Red blood cells (54) Infants with congenital syphilis might have up to 500 NRBC/100 WBCs, most likely resulting from the presence of active hemolysis and extramedullary hemopoieses (55)

ACUTE STRESS

Acute and subacute asphyxia

"Boskabadi et al reported that Nucleated Red blood cells count is increased in new-born with perinatal asphyxia (mean 18.63/100 WBC) when compared to normal new-born(mean 3.87/100 WBC) and also demonstrated a positive correlation between markers of severity of perinatal asphyxia and Nucleated Red blood cells count(56)." It is a common misconception that only long standing conditions cause raised circulating Nucleated Red blood cells at birth but acute and subacute stress can also cause such increases(57) Interestingly, even the relative hypoxia of normal labour without asphyxia has been associated with increased cord erythropoietin levels and Nucleated Red blood cells compared with samples from infants born by elective caesarean section without labour(58)

"In 1970, Merenstein et al reported raise in Nucleated Red blood cells in the blood of infants within 6 hours of delivery after acute intrapartum asphyxia(59) Various other studies have confirmed the finding of increased Nucleated Red blood cells in cord blood and neonatal blood following acute asphyxia(14,56,58,60,61)"

"Thilaganathan et al found important variations in cord Nucleated Red blood cells of new-born babies by emergency caesarean section (median = 1100 NRBC/mm3) compared with new-born babies by elective caesarean section (median = 300 NRBC/

mm3)(58) But, there was important overlap between the groups: in some infants born by emergency caesarean section no Nucleated Red Blood cells were detected, and some infants born by elective caesarean section had large numbers of Nucleated Red Blood cells."

"Naeye and Localio compared sixteen term and preterm new-borns who developed cerebral palsy following acute birth asphyxia with seven new-born having long standing developmental disorders unrelated to a perinatal insult, and also with 84 normal controls (62) Few normal controls had nucleated red blood cells values surpassing 2000 NRBC/mm3. All of the infants with cerebral palsy caused by developmental events unrelated to birth had less than 2000 NRBC/mm3. Nucleated red blood cells had raised to 2000/mm3 or more in 15 of the 16 new-borns who had acute ischemia and hypoxemia. The magnitude of the rise in nucleated red blood cells following acute asphyxia could be a function of both the severity and length of the asphyxia."

"Hanlon-Lundberg and Kirby evaluated the relation between the severity of asphyxia and increased nucleated red blood cells by comparing cord nucleated red blood cells with cord pH and Apgar scores" (14) The nucleated red blood cells counts increased with progressive increases in cord metabolic acidosis and with progressive decreases in the Apgar scores. However, not all new-borns with low Apgar scores had increased nucleated red blood cells; in some new-borns with very low Apgar scores, almost no nucleated red blood cells were detected, and other new-borns with normal Apgar scores had as several as 2250 NRBC/mm3. Similarly, some new-borns with a pH < 7.00 had as few as 260NRBC/mm3, where as others with normal cord pH values had significantly

increased nucleated red blood cells. Different investigators have also found increased nucleated red blood cells associated with a fall incord pH (57,58,59)"

"Korst et al and Phelan et al evaluated the relation between the duration of asphyxia and increases in Nucleated Red blood cell." (60,61) Infants with a persistent non reactive fetal heart rate pattern from admission to delivery were presumed to havesuffered a more long-standing, subacute, asphyxia episode. Samples from these infants were compared with values from infants who had suffered acute intrapartum asphyxia, often from a catastrophic event such as a cord prolapse or a ruptured uterus Both groups had significantly increased Nucleated Red blood cell counts compared with historical controls. Although infants with the subacute asphyxia had higher Nucleated Red blood cell counts, there was much over lap between the groups: some infants with subacute injury had noNucleated Red blood cell and other infants in the acute group had as many as 11476 NRBC/mm3. The infants with preadmission injury had longer Nucleated Red blood cell clearance times than the acuteinjury group, but again there was a large overlap between the two groups. The data appears to show that the clearance rate for the groups was similar, the preadmission group merely beginning with higher values and therefore requiring longer clearance times. These studies did not indicate the severity of the asphyxia of the two groups. It remains possible that the group with subacute injury was more severely asphyxiated than the group with acute injury, in which case the difference in Nucleated Red blood cell may, in part,reflect the increased severity of asphyxia rather than solely be attributable to increased duration of asphyxia.The precise time required to observe an increase in circulating Nucleated Red blood cell in the new-

born is not known. Atshuler and Hyder found that Nucleated Red blood cell increased to 2000/mm3within two hours of acute blood loss in previously healthy term fetuses. (63)

Benirschke reported a new-born with an NRBC response detectable within one hour of an acute hypoxic event.(64) Fanaroff concluded that normoblasts could enter the bloods tream within 30 minutes of a severe hypoxic injury(65) Naeye reported finding NRBC "in large numbers" 20 minutes after the start of neonatal hypoxia(66) Korst et al and Phelanet al found increased NRBC after acute catastrophic intrapartum events(60,61) The duration of these catastrophic events was undoubtedly less than one hour in mos tcases. Future studies using fetal scalp samples and cord blood at birth may be use ful in determining the time necessary for the rise to be detected, although it is now reasonable to conclude that it is less than 60 minutes and perhaps as short as 20 to 30 minutes. Similarly, the time interval between erythropoietin rise and peak erythrocyte count is unknown, most evidences in humans suggests that it takes at least 24 to 48hours (representing no preformed source) and declines by seven days.

Acute Chorioamnionitis

Acute chorioamnionitis has been associated with increased levels oferythropoietin and increased new-born NRBC. Maier et al found significantly elevated erythropoietin levels in neonates whose placentas showed signs of chorioamnionitis. (67)Salafia et al speculated that the increase in NRBC may be a fetal response to an inflamed environment and not due to fetal Hypoxia & Increased NRBC have been reported in preterm infants born after pregnancies complicated by chorioamnionitis without cord acidosis or hypoxaemia (68). Leikin et al found an increase in NRBC when histological chorioamnionitis was present without signs of clinical Chorioamnionitis (69)

Postnatal Hypoxia

If acute hypoxia during labour can lead to increased NRBC within minutes orhours of birth, it would be expected that postnatal hypoxia could also lead to a rapid release of NRBC. Indeed, infants with severe pulmonary disease and cyanotic heart disease have elevated erythropoietin levels during the first week of life (70). Naeye and Localio reported infants with severe hypoxemia resulting from pneumonia or cyanotic congenital heart disease had NRBC counts in excess of 2000/mm3(62) Infants with congenital diaphragmatic hernias may have increased NRBC within 20 minutes of birth, presumably the result of postnatal marrow release (66)

Idiopathic

About one to two percent of apparently normal new-born have idiopathic increase in NRBC. Hanlon-Lundberg et al examined cord blood NRBC in 1112 term new-born (51) Nine (0.8%) hada count greater than 100 NRBC/100WBCs. There was no apparent cause for the increase in eight of the nine; these eight had uneventful antepartum, intrapartum, and neonatal courses. Naeye and Localio reported finding two (2.4%) “outliers” among 84 normal term infants (62) One of these two had 12444 NRBC/mm3. Green and Mimouni found that five percent of 102 normal controlinfants had absolute NRBC counts greater than 1700 /mm3(50)

Clearance of NRBC

It is difficult to precisely predict the time required for NRBC to clear from the peripheral circulation of the new-bor baby as various studies show variable time frame.

But growing body of evidence narrows this time to 72 to 96 hours. In a study conducted by Phelan et al, the time required to clear NRBC in the babies with perinatal asphyxia was 236 ± 166 hours in comparison to 56 ± 37 hours in normal term infants (71). In another study conducted by Merenstein et al, the time required to clear NRBC in healthy term babies was 48 hours while that in case of babies with perinatal asphyxia it was greater than 72 hours (59).

Units of Reporting

Clinically it is best to express NRBC as an absolute number of cells per unit volume, either "NRBC/mm3" or "NRBC/L". However, due to lack of sophisticated counters required for counting absolute number of NRBC, most clinical laboratories and many research publications report NRBC relative to 100 white blood cells (WBCs)(16). Unfortunately, the extreme variability in the number of leucocytes after birth results in a wide range of values for NRBC when they are expressed relative to the WBC count. Many pathological processes that significantly alter the total leukocyte count magnify the problem (17). Processes that increase the leukocyte count will result in a misleadingly low value of NRBC when reported relative to WBCs, and processes that decrease the leukocyte count will produce misleadingly high NRBC counts if reported relative to WBCs. Data dispersion is presented as the mean ± 1 standard deviation (SD).

Normal new-born Values

Since Lippman, many investigators have reported values of NRBC at and shortly after the birth.

REFERENCE	SAMPLE SIZE	NRBC	AGE	GESTATION/ BIRTH WEIGHT
Naeye(61)	84	919±1425 nrbc/mm^3	1 hour	term
Green(49)	102	400±1300 nrbc/mm^3	12-24 hours	37-41 weeks AGA
Sinha(241)	84	2.3 ± 0.7 NRBC/100WBCs	Birth (Cord blood)	2501-3500 grams
Shivhare(242)	33	4.1 ± 2.4 NRBC/100WBCs	Birth (Cord blood)	Term and near term
Phelan(234)	83	3.4 ± 3.0 NRBC/100WBCs	Birth (Cord blood)	≥ 37 weeks, > 2700 gms
Hanlon-lundberg(244)	1112	8.5 ± 10.3 NRBC/100WBCs	Birth (Cord blood)	37 – 41 weeks*
Buonocore(15)	105	1689 ± 290 NRBC/100 WBCs	Birth (Cord blood)	37 – 41 weeks*
Axt(245)	304	3.7 (median) NRBC/100 WBCs	Birth (Cord blood)	261 – 289 days
		6.5 (median) NRBC/100 WBCs	Birth (Cord blood)	290 + days

Table no 2: Normal New born values of NRBC in various studies

Results are mean ±1 SD.

*Excludes eight infants with extreme idiopathic increases (>100 NRBC/100 WBCs) and includes infants with maternal diabetes, growth retardation, birth asphyxia, and other causes known to increase the circulating NRBC

AGA – Appropriate size for gestational age

WBC – White blood cells

It is reasonable to conclude that the mean value of NRBC in the first few hours of life in healthy term new-born is about 500 NRBC/mm3, and that a value above 1000 NRBC/mm3 can be considered elevated (36,53,246). Expressed differently, 0 to 10 NRBC/ 100 WBCs are typical, and values above 10 to 20 NRBC/100 WBCs are elevated, although these values are highly dependent on the total leukocyte count. Studies have consistently shown decreasing NRBC as the gestational age increases, except that post-term infants have higher counts than term infants, secondary to increased incidence of fetal acidosis in post-term pregnancies (245,247-249). Small premature new-born may normally have up to 10,000 NRBC/mm3(250). In the normal neonate, NRBC are rapidly cleared from the bloodstream after birth (6,61,53). By 12 hours of age, the counts fall by about 50%, and by 48 hours only 20 to 30 NRBC/mm3 are found. In healthy term new-born, virtually no NRBC are found after the third or 12 fourth day of life, although they may persist in small numbers up to one week in preterm new-born (53,250).

METHODOLOGY

METHODOLOGY

Source of data:

All babies Satisfying Inclusion criteria in NICU and PNC Ward of SHRI BM PATIL MEDICAL COLLEGE & RESEARCH CENTRE.

- Case with perinatalasphyxia admitted in NICU and
- controls are normal neonates in postnatal ward.

Study design: Prospective Observational Comparative study

Sample size: Minimum of 125 cases or more of Birth Asphyxia and equal number of controls studied in a span of 1.5 year.

Sampling Methods: Purposive sampling

Selection criteria

1. **Inclusion criteria:**

All term /IUGR babies

- Inborn Babies admitted with Apgar below 7 at 1min or
- Out born Babies admitted with H/o No cry/Delayed cry in NICU at,

  Shri B. M. Patil Medical College, Hospital & Research Center, Vijayapur.
- Normal term Babies in post-natal ward(controls)

2. **Exclusion criteria:**

The study has excluded

- Birth weight less than 1500 gm or Gestational age below 37 weeks.
- Syndromic Babies, Abnormal babies (eg: Hydrops) or babies with multiple anomalies or single major anomaly like any congenital cardiac, renal anomaly and congenital infections.

- Opium or Anesthesia or any other drug to mother related Low APGAR Score.

Method of collection of data:

Consent:

Before enrolling the baby in the study an informed consent of the parents was taken after explaining in detail about the methods and procedures involved in the study in their vernacular language.The study was approved by the Institutional Ethics Committee of SHRI BM PATIL MEDICAL COLLEGE & RESEARCH CENTRE, VIJAYAPUR

Enrolment:

Details of the maternal parameters like age, past obstetric history, present pregnancy, medical history, medications taken during pregnancy, details of labour and delivery were recorded in a proforma. Details of the baby like date of birth, sex, gestational age, birth weight, Apgar score at one and five minutes, resuscitation (American Academy of Paediatrics guidelines) and examination details were recorded. Clinical assessments include assessments of the neurologic status daily during the stay, the grade of HIE (Stage I, Stage II or Stage III), the type of respiratory support needed, the presence of seizures and involvement of multiorgan dysfunction. The time taken for establishment of full oral feedings through sucking, time taken for recovery and duration of hospital stay and neurologic examination at discharge were also noted. Laboratory assessments include CBC, Nucleate RBC counts, Reticulocyte counts and routine investigations for Birth Asphyxia as per NICU protocol. Gestational age was assessed by using New Ballard's scoring. Hypoxic ischemic encephalopathy if present was graded using Sarnat and Sarnat staging.

Laboratory Investigation:

Two ml of Cord blood for Inborn Babies and Peripheral venous blood was collected within 6 hours of birth in ethylene diamine tetra acetate bulbs from both cases and controls. Samples were stored in refrigerator if there was any delay in processing. Blood samples were used for making smears (for NRBCs) and complete blood count. Babies with birth asphyxia were further admitted in NICU and investigated as per the routine NICU protocol for birth asphyxia. Samples were processed and analysed by the same blinded pathologist. The ethylene diamine tetra acetate sample was processed by **SYSMEX-XN 1000** for obtaining total white cell count and platelet count.

Figure no 2: Sysmex XN-1000™ Hematology Analyzer

The blood smears were stained by Leishman stain and manual differential count was done to count NRBCs, and Reticulocyte count. Number of nucleated red blood cells were counted per 100 leukocytes in peripheral smears and were reported as 'number of NRBC/100 WBC' and reticulocyte counts were reported in percentage.

Outcome measures:

- Discharge with no sequelae
- Discharge with sequelae
- Death/DAMA
- Duration of NICU stay

Statistical analysis:

All characteristics were summarized descriptively. For continuous variables, the summary statistics of mean, standard deviation (SD) were used. For categorical data, the number and percentage were used in the data summaries. Chi-square (χ^2)/ Freeman-Halton Fisher exact test was employed to determine the significance of differences between groups for categorical data. The difference of the means of analysis variables between two independent groups was tested by unpaired t test. If the p-value was < 0.05, then the results were considered to be statistically significant otherwise it was considered as not statistically significant. Data were analyzed using SPSS software v.23.0. and Microsoft office.

RESULTS

RESULTS

The present study was conducted from 2nd November 2016 to 31st July 2018 among 250 term neonates (125 cases and 125 controls) fulfilling the preformed inclusion criteria, at NICU, Shri B. M. Patil Medical College, Hospital & Research Center, Vijayapur.

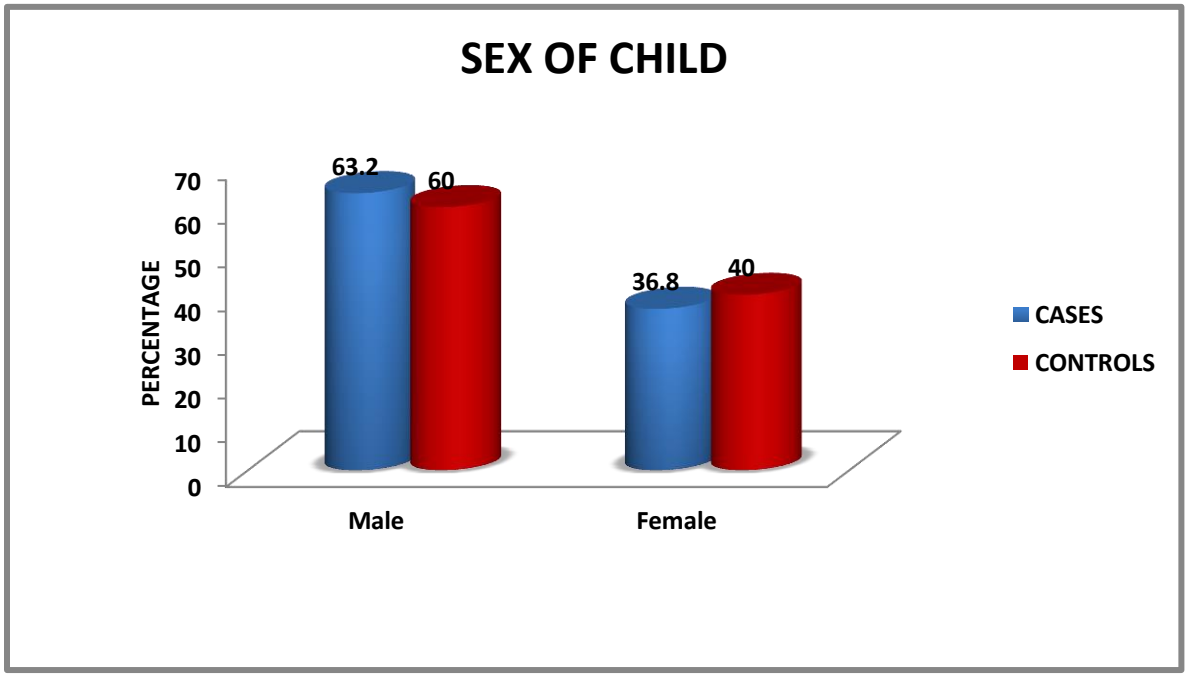
PATIENT DEMOGRAPHY

1. Distribution of sex of child between cases and control

TABLE 3: distribution of sex of child between cases and control

SEX OF CHILD	CASES		CONTROLS		p value
	N	%	N	%	
Male	79	63.2	75	60	0.603
Female	46	36.8	50	40	
Total	125	100	125	100	

FIGURE 3: distribution of sex of child between cases and control



The two study groups were compared between cases and controls in terms of sex distribution. Table no 3 shows that Sex distribution have no statistical significance.

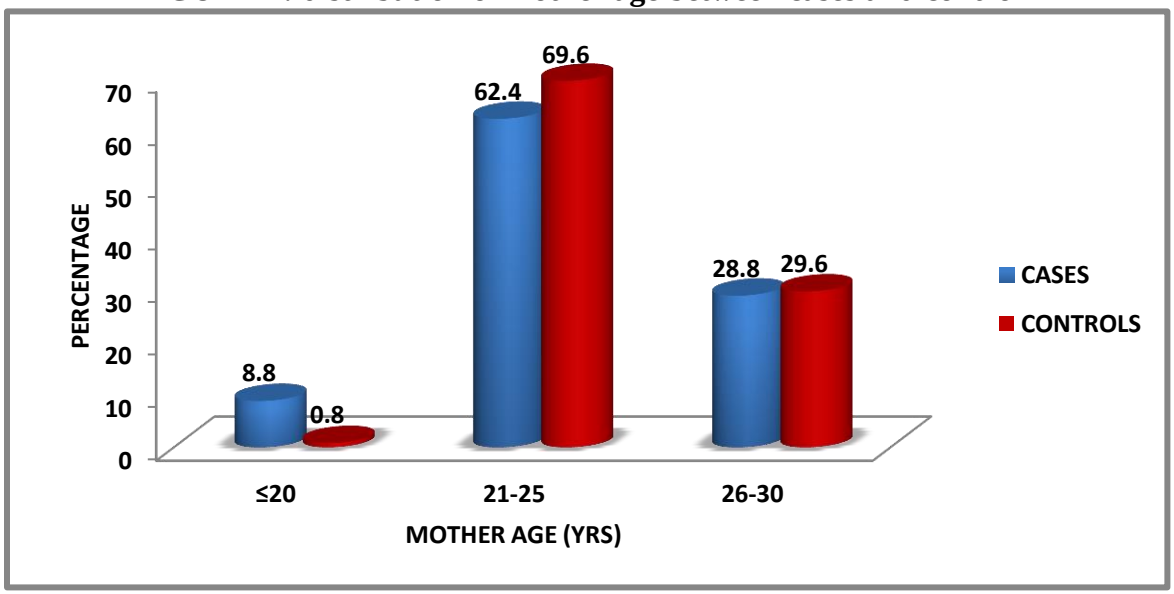
2. Distribution of mother age between cases and control

TABLE 4: distribution of mother age between cases and control

MOTHER AGE	CASES		CONTROLS		p value
	N	%	N	%	
≤20	11	8.8	1	0.8	0.012*
21-25	78	62.4	87	69.6	
26-30	36	28.8	37	29.6	
Total	125	100	125	100	

Note: * significant at 5% level of significance (p<0.05)

FIGURE 4: distribution of mother age between cases and control



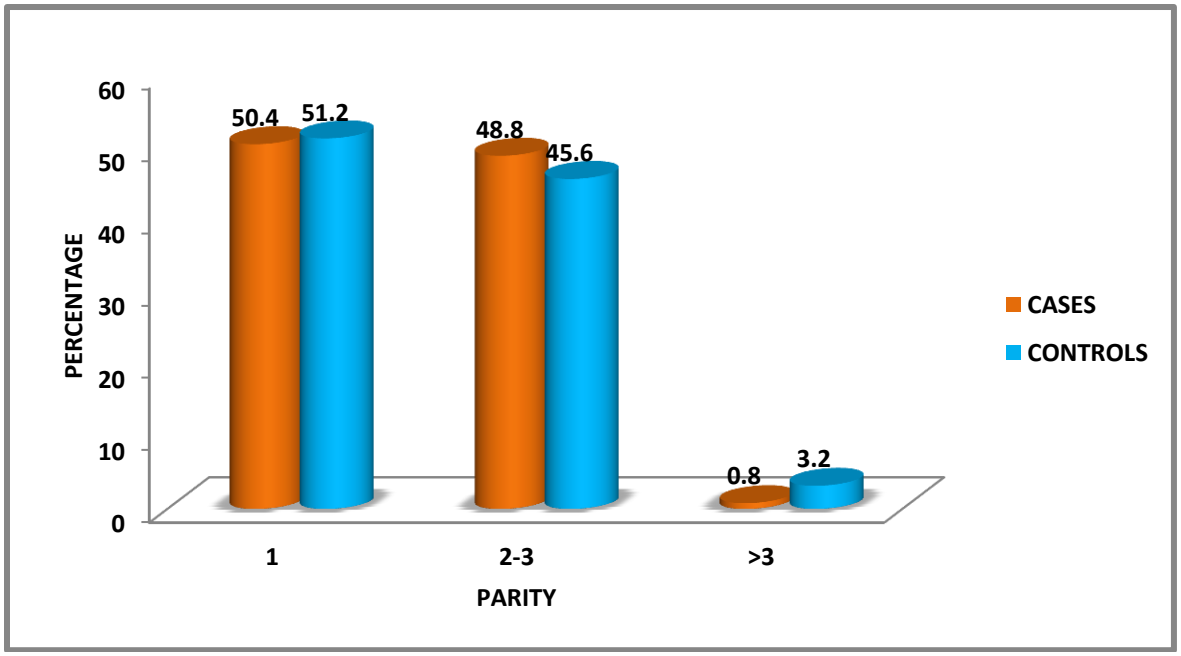
The two study groups were compared between cases and controls in terms of Mothers age. Table no 4 shows that mothers of age <20 years were 8.8% in cases and 0.8% in controls and age between 21-25 years were 62.4% in cases and 69.6% in controls and age between 26-30 years were 28.8% in cases and 29.6% in controls. And as we can see the degree of association of distribution of mothers age between cases and control is statistically significant.

3. Distribution of parity between cases and control

TABLE 5: distribution of parity between cases and control

PARITY	CASES		CONTROLS		p value
	N	%	N	%	
1	63	50.4	64	51.2	0.378
2-3	61	48.8	57	45.6	
>3	1	0.8	4	3.2	
Total	125	100	125	100	

FIGURE 5: distribution of parity between cases and control



The two study groups were compared between cases and controls in terms of parity. Table no 5 shows that primigravida mothers were 50.4% in cases and 51.2% in controls and Gravida 2 and 3 were 48.8% in cases and 45.6% in controls and gravida >3 were 0.8% in cases and 3.2% in controls. And as we can see the degree of association of distribution of Parity between cases and control is statistically not significant.

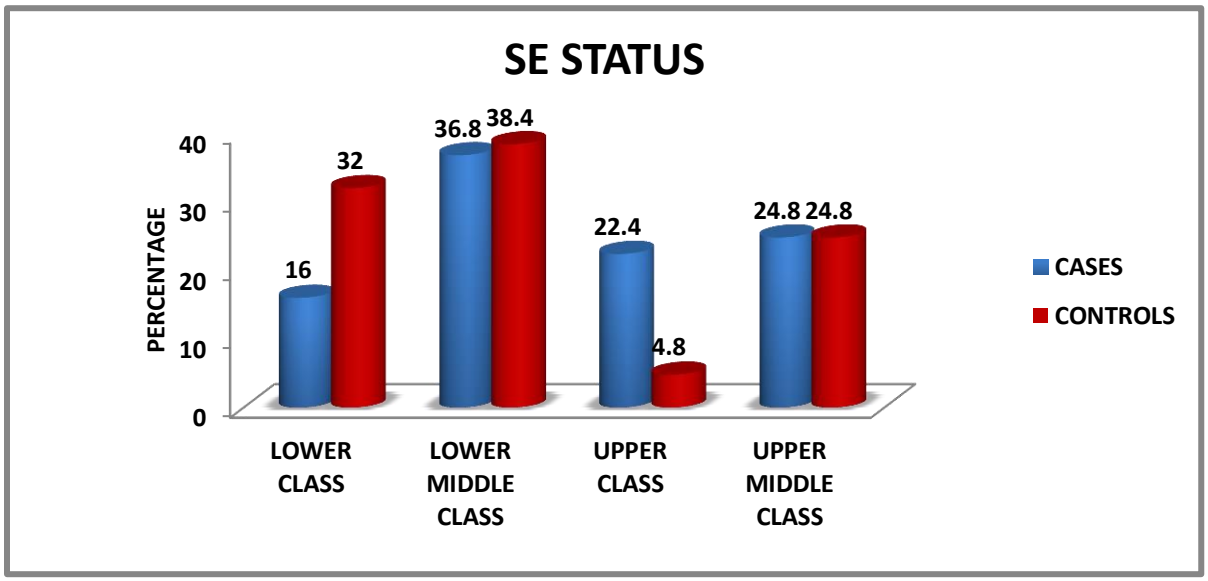
4. Distribution of se status between cases and control

TABLE 6: distribution of se status between cases and control

SE STATUS (MODIFIED KUPPUSWAMY SCALE)	CASES		CONTROLS		p value
	N	%	N	%	
LOWER CLASS	20	16	40	32	<0.001 *
LOWER MIDDLE CLASS	46	36.8	48	38.4	
UPPER CLASS	28	22.4	6	4.8	
UPPER MIDDLE CLASS	31	24.8	31	24.8	
Total	125	100	125	100	

Note: * significant at 5% level of significance (p<0.05)

FIGURE 6: distribution of se status between cases andControl



The two study groups were compared between cases and controls in terms of Socio economic status. Table no 6 shows that Low SES were 16% in cases and 32% in controls Lower middle class were 36.8% in cases and 38.4% in controls and Upper middle class were 24.8% in cases and 24.8% in controls and Upper class were 22.4% in cases and 4.8% in controls. And as we can see the degree of association of distribution of SES between cases and control is statistically significant.

MATERNAL RISK FACTORS

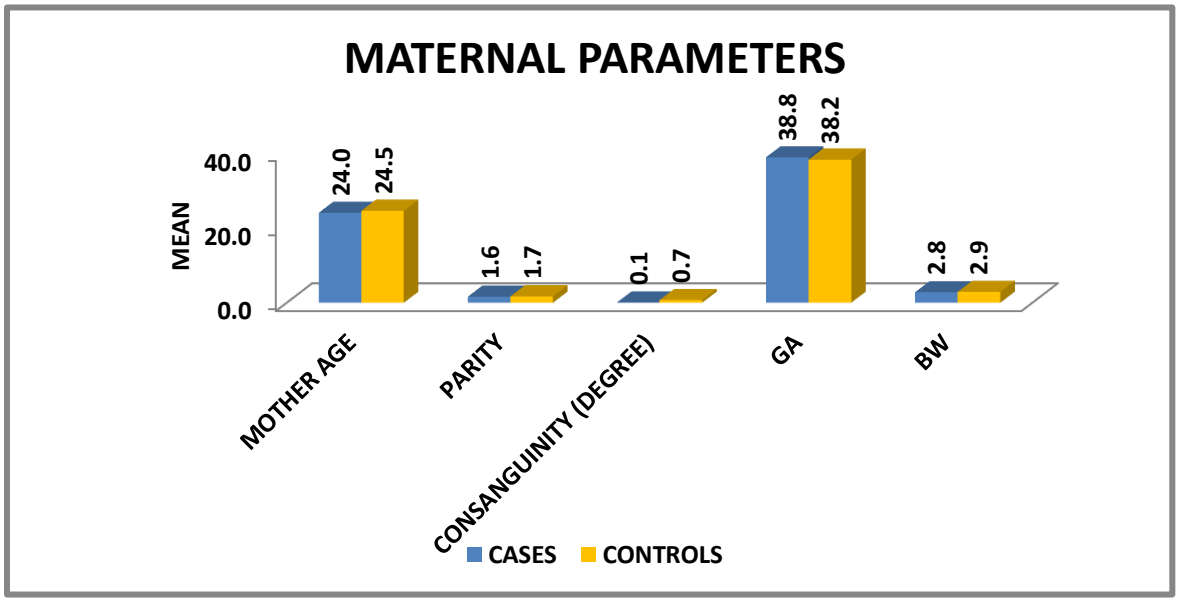
1. **Comparison of maternal parameters between cases and control**

TABLE 7: comparison of maternal parameters between cases and control

MATERNAL PARAMETERS	CASES		CONTROLS		p value
	Mean	SD	Mean	SD	
MOTHER AGE	24.0	2.3	24.5	1.7	0.032
PARITY	1.6	0.7	1.7	0.9	0.525
CONSANGUINITY (DEGREE)	0.1	0.4	0.7	1.2	<0.001*
GA	38.8	1.3	38.2	1.1	<0.001*
BW	2.8	0.4	2.9	0.2	0.016

Note: * significant at 5% level of significance (p<0.05)

FIGURE 7: comparison of maternal parameters between cases and control



The two study groups were compared between cases and controls in terms of Maternal parameters. Table no 7 shows Mean mothers age, Parity and Birth weight have no statistical significance but Consanguinity and Gestational age have statistical significance.

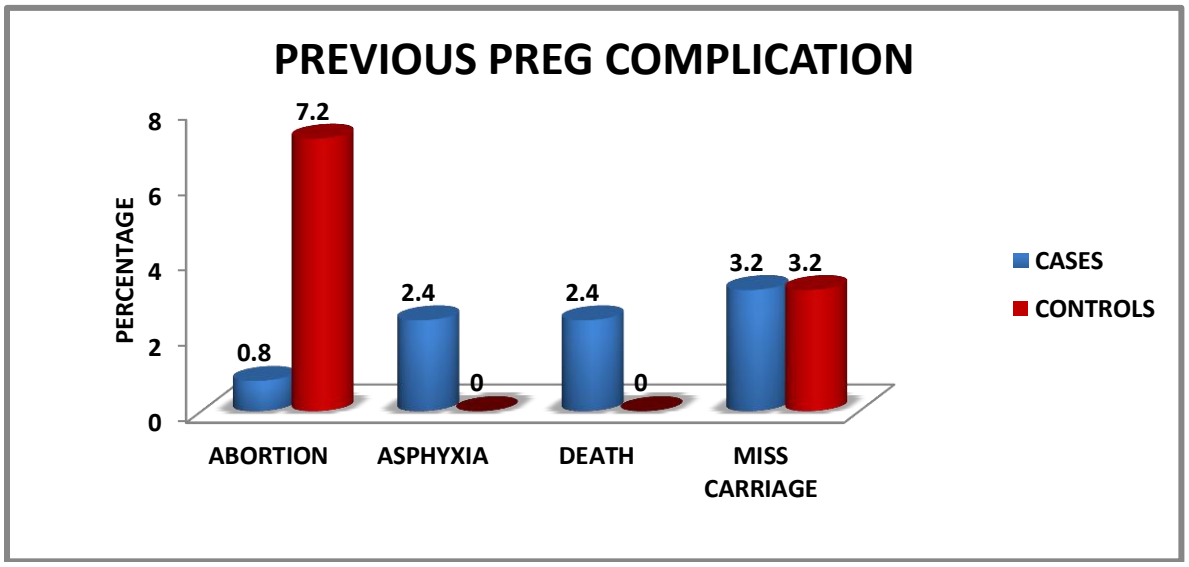
2. Distribution of previous preg complication between cases and control

TABLE 8: distribution of previous preg complication between cases and control

PREVIOUS PREG COMPLICATION	CASES		CONTROLS		p value
	N	%	N	%	
ABORTION	1	0.8	9	7.2	0.015*
ASPHYXIA	3	2.4	0	0	
DEATH	3	2.4	0	0	
MISS CARRIAGE	4	3.2	4	3.2	
NIL	114	91.2	112	89.6	
Total	125	100	125	100	

Note: * significant at 5% level of significance (p<0.05)

FIGURE 8: distribution of previous preg complication between cases and control



The two study groups were compared between cases and controls in terms of Previous pregnancy complications. Table no 8 shows that previous pregnancy complications like abortions, Asphyxia, Previous death of baby, Past miscarriages have statistical significance.

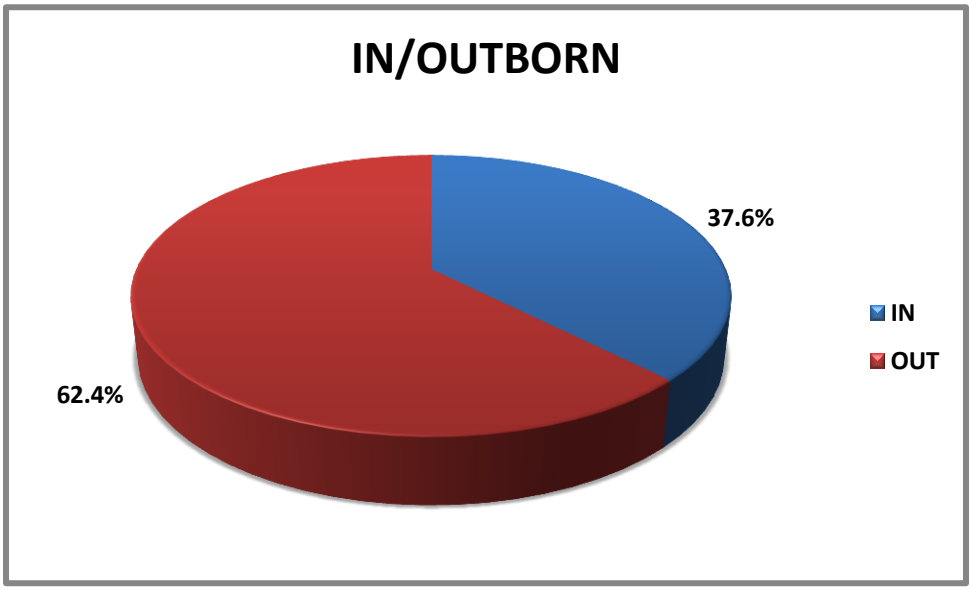
NEONATAL FACTORS

1. Distribution of IN/OUTBORN babies among cases

TABLE 9: distribution of IN/OUTBORN babies among cases

IN/OUTBORN	N	%
IN	47	37.6
OUT	78	62.4
Total	125	100

FIGURE 9: distribution of in/outborn babies among cases



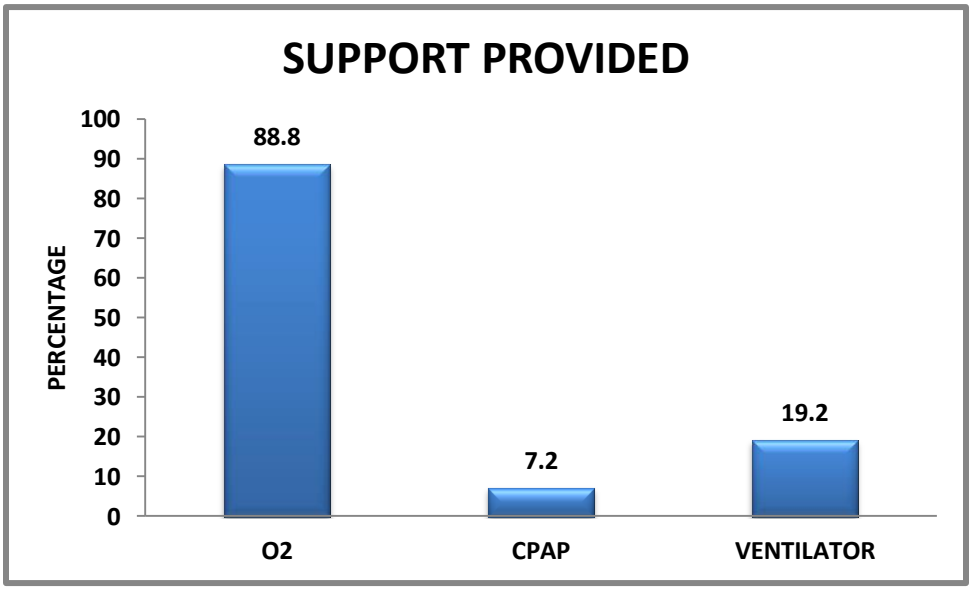
In our study group among cases 37.6% of babies with asphyxia were born in our hospital and 62.4% of babies with asphyxia were referred from other hospitals.

2. Support provided among cases

TABLE 10: support provided among cases

SUPPORT GIVEN	N	%
O2	111	88.8
CPAP	9	7.2
VENTILATOR	24	19.2

FIGURE 10: support provided among cases



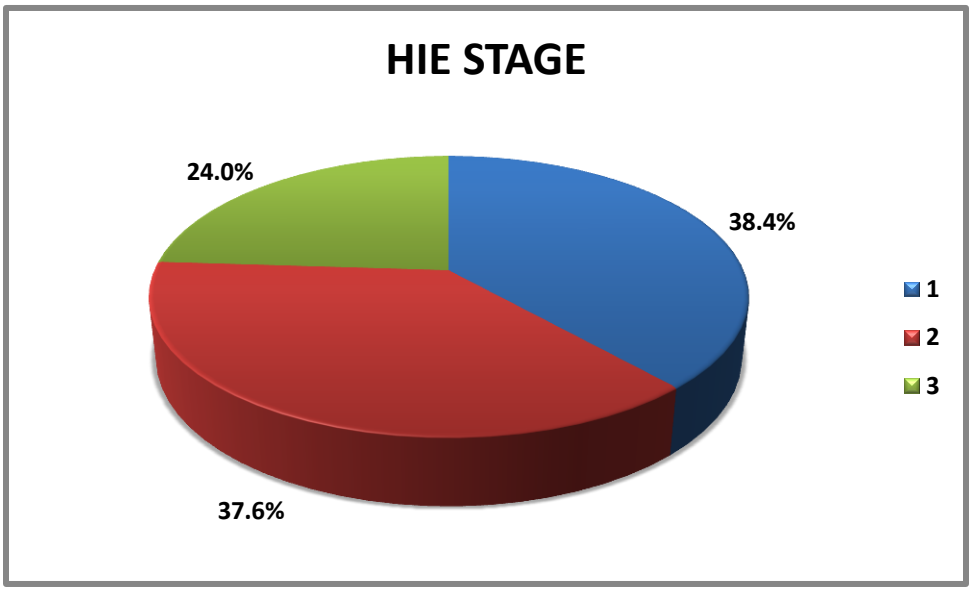
In Our study group among cases 19.2% of babies with asphyxia were Mechanically ventilated, 7.2% of babies with asphyxia needed CPAP support and 88.8% of babies with asphyxia were managed with Oxygen support.

3. Distribution of HIE stage among cases

TABLE 11: distribution of HIE stage among cases

HIE STAGE	N	%
1	48	38.4
2	47	37.6
3	30	24
Total	125	100

FIGURE 11: distribution of HIE stage among cases



In Our study group among cases 38.4% of babies with asphyxia belonged to HIE stage 1, 37.6% of babies with asphyxia belonged to HIE stage and 24% of babies with asphyxia belonged to HIE stage 3, according to Sarnat & Sarnat staging of asphyxiated babies.

HEMATOLOGICAL MARKERS

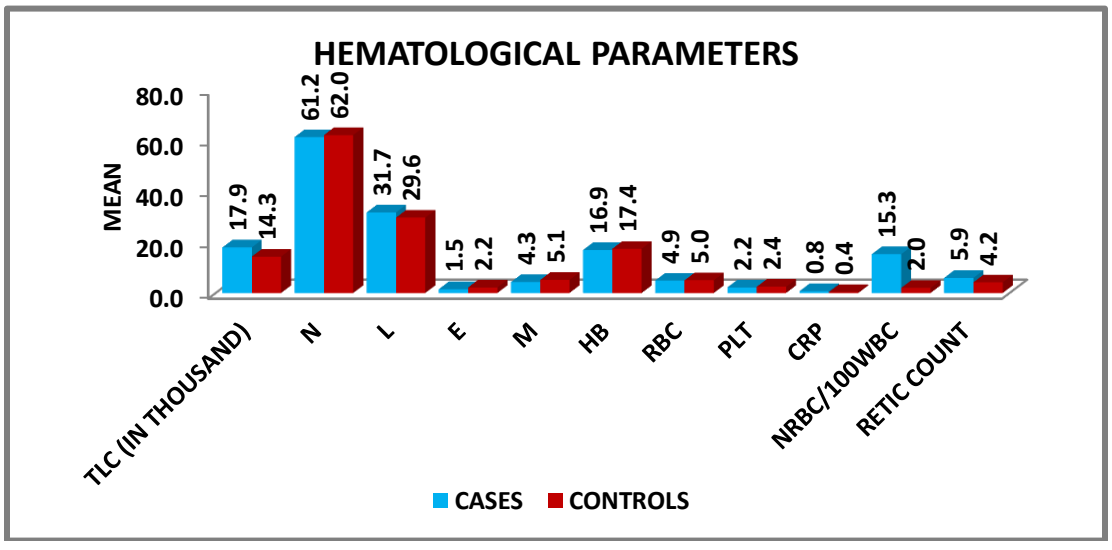
1. **Distribution of hematological parameters among cases**

TABLE 12: distribution of hematological parameters among cases

HEMATOLOGICAL PARAMETERS	CASES		CONTROLS		p value
	Mean	SD	Mean	SD	
TLC	17901.6	7829.2	14308.3	6700.8	<0.001*
N	61.2	14.1	62.0	15.8	0.684
L	31.7	13.3	29.6	14.5	0.242
E	1.5	2.0	2.2	2.8	0.03
M	4.3	2.5	5.1	2.8	0.012
HB	16.9	2.9	17.4	2.7	0.16
RBC	4.9	1.0	5.0	0.8	0.34
PLT	2.2	0.9	2.4	0.9	0.046
CRP	0.8	0.9	0.4	0.3	<0.001*
NRBC/100WBC	15.3	21.3	2.0	3.0	<0.001*
RETIC COUNT	5.9	2.8	4.2	2.0	<0.001*

Note: * significant at 5% level of significance (p<0.05)

FIGURE 12: distribution of hematological parameters among cases



The two study groups were compared between cases and controls in terms of Hematological parameters. Table no 12 shows that Total Count, C-reactive Protein, NRBC/100 WBCs and Retic Counts have statistical significance, while Differential counts, Hemoglobin, RBC and Platelet counts have no statistical significance.

APGAR SCORE

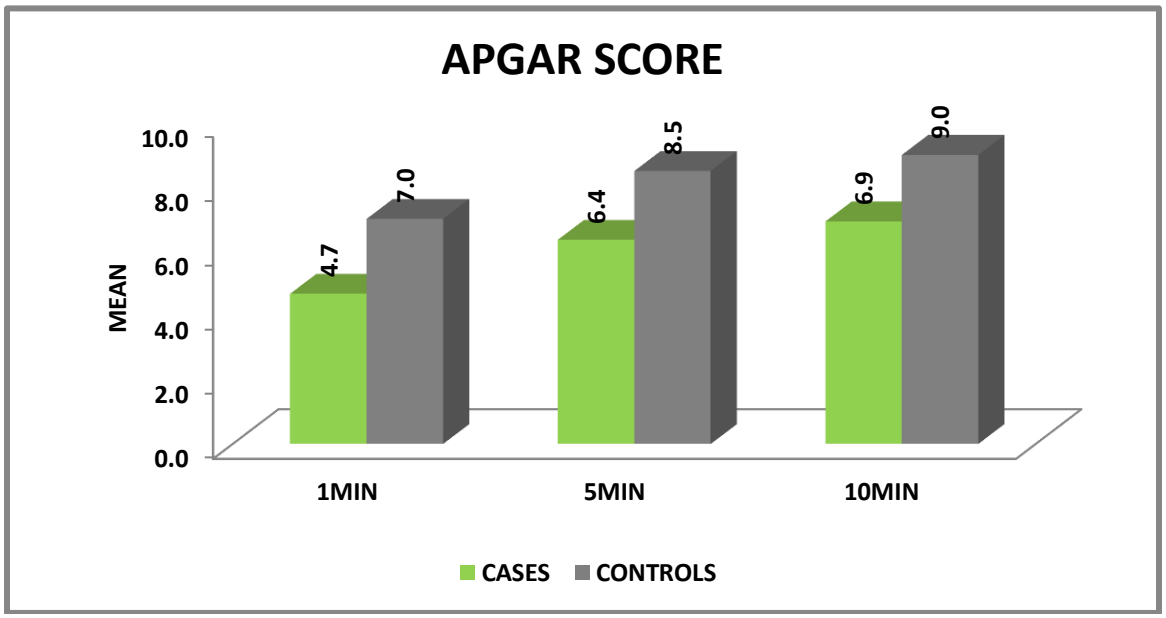
Mean APGAR score between cases and control

TABLE 13: mean APGAR score between cases and control

APGAR SCORE	CASES		CONTROLS		p value
	Mean	SD	Mean	SD	
1MIN	4.7	1.2	7.0	0.0	<0.001*
5MIN	6.4	1.0	8.5	0.5	<0.001*
10MIN	6.9	0.8	9.0	0.1	<0.001*

Note: * significant at 5% level of significance (p<0.05)

FIGURE 13: mean APGAR score between cases and control



The two study groups were compared between cases and controls in terms of Mean APGAR score. Table no 13 shows that mean APGAR scores were 7 at 1st min, 8.5 at 5th min and 9 at 10th min in controls whereas 4.7 at 1st min, 6.4 at 5th min and 6.9 at 10th min in cases which have statistical significance.

RETICULOCYTE COUNT

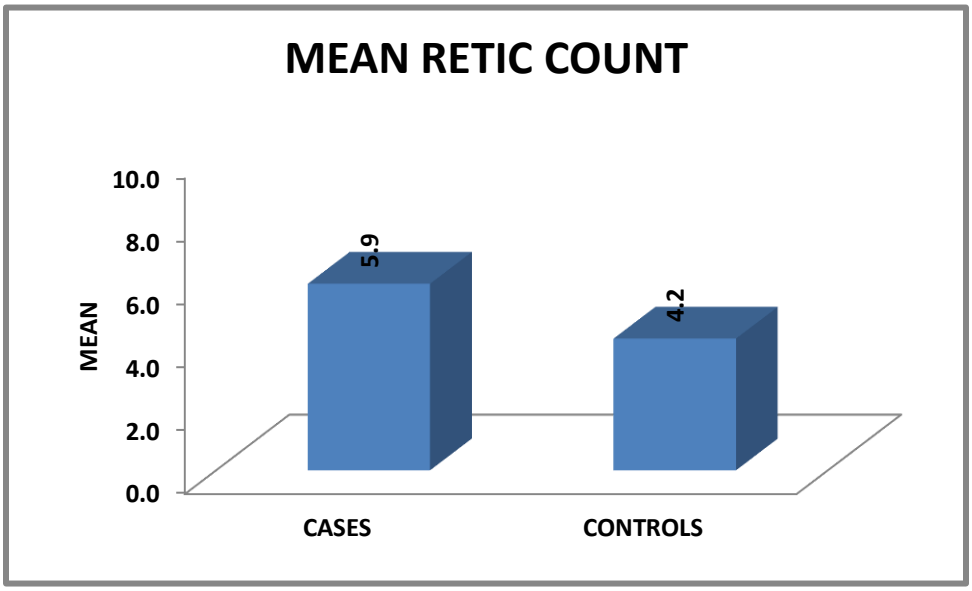
1. Mean Retic Count among cases and controls

TABLE 14: mean Retic Count among cases and controls

RETIC COUNT	CASES		CONTROLS		p value
	Mean	SD	Mean	SD	
	5.9	2.8	4.2	2.0	<0.001*

Note: * significant at 5% level of significance (p<0.05)

FIGURE 14: mean Retic Count among cases and controls



In the above table we can see the mean reticulocyte count among cases and controls and can conclude that the mean reticulocyte count is more and is statistically significant.

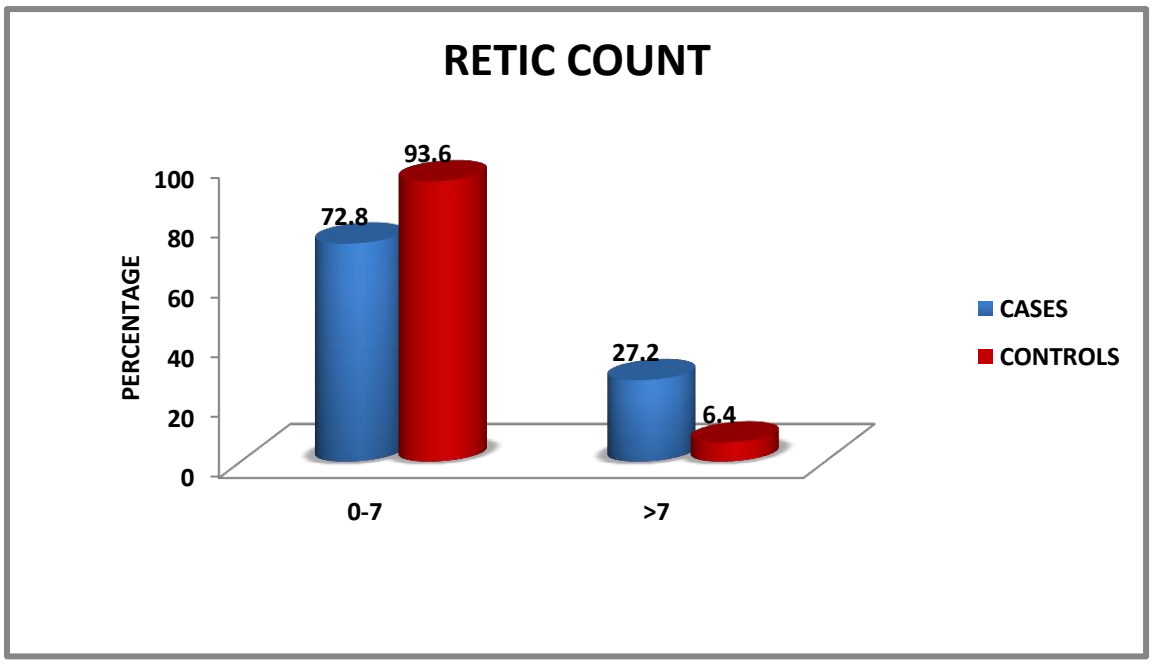
2. Distribution of Retic Count between cases and control

TABLE 15: distribution of retic count between cases and control

RETIC COUNT	CASES		CONTROLS		p value
	N	%	N	%	
0-7	91	72.8	117	93.6	<0.001*
>7	34	27.2	8	6.4	
Total	125	100	125	100	

Note: * significant at 5% level of significance (p<0.05)

FIGURE 15: distribution of Retic Count between cases and control



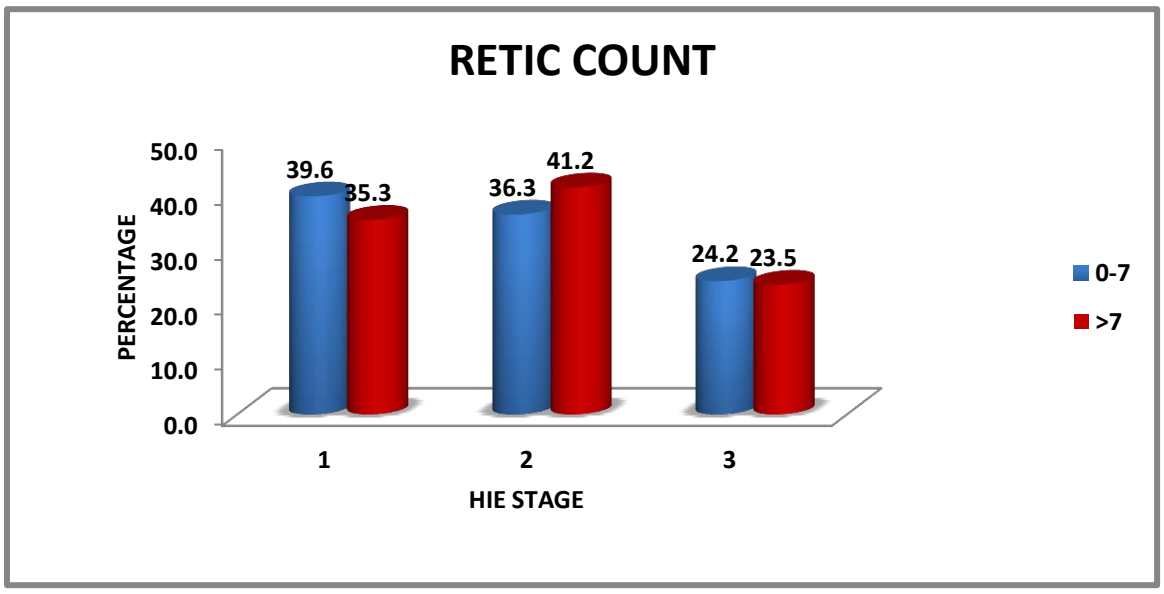
The two study groups were compared between cases and controls in terms of Reticulocyte count. Table no 15 shows that Reticulocyte counts of 0-7% were more in controls whereas >7% were more in cases which have statistical significance.

3. Distribution of Retic Count according to HIE stage

TABLE 16: distribution of Retic Count according to HIE stage

HIE STAGE	RETIC COUNT				p value
	0-7		>7		
	N	%	N	%	
1	36	39.6	12	35.3	0.869
2	33	36.3	14	41.2	
3	22	24.2	8	23.5	
Total	91	100.0	34	100.0	

FIGURE 16: distribution of Retic Count according to HIE stage



The two study groups were compared between cases and controls in terms of distribution of Retic count according to HIE stage. Table no 16 shows that distribution of reticulocyte count and HIE staging have No statistical significance.

NUCLEATED RBC'S

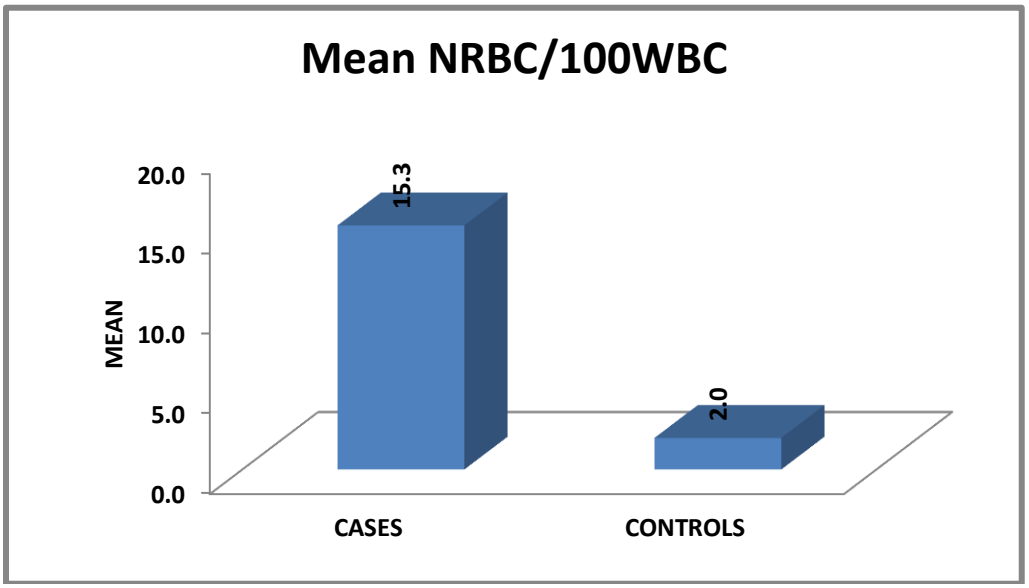
1. Mean NRBC/100WBC among cases and controls

TABLE 17: mean NRBC/100WBC among cases and controls

NRBC/100WBC	CASES		CONTROLS		p value
	Mean	SD	Mean	SD	
	15.3	21.3	2.0	3.0	<0.001*

Note: * significant at 5% level of significance (p<0.05)

FIGURE 17: mean NRBC/100WBC among cases and controls



In the above table we can see the mean nRBC count among cases and controls and can conclude that the mean nRBC count is more and is statistically significant.

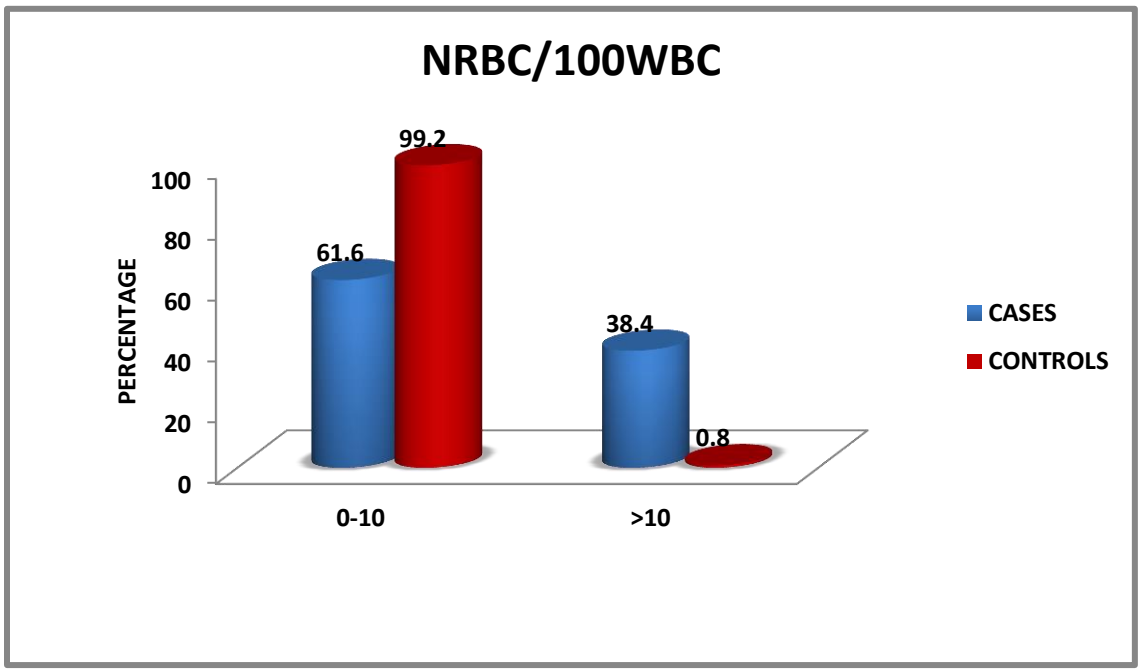
2. Distribution of NRBC/100WBC between cases and control

TABLE 18: distribution of NRBC/100WBC between cases and control

NRBC/100WBC	CASES		CONTROLS		p value
	N	%	N	%	
0-10	77	61.6	124	99.2	<0.001*
>10	48	38.4	1	0.8	
Total	125	100	125	100	

Note: * significant at 5% level of significance (p<0.05)

FIGURE 18: distribution of NRBC/100WBC between cases and control



The two study groups were compared between cases and controls in terms of Nucleated RBC count. Table no 18 shows that nucleated RBC counts of 0-10 were more in controls whereas >10 were more in cases which have statistical significance.

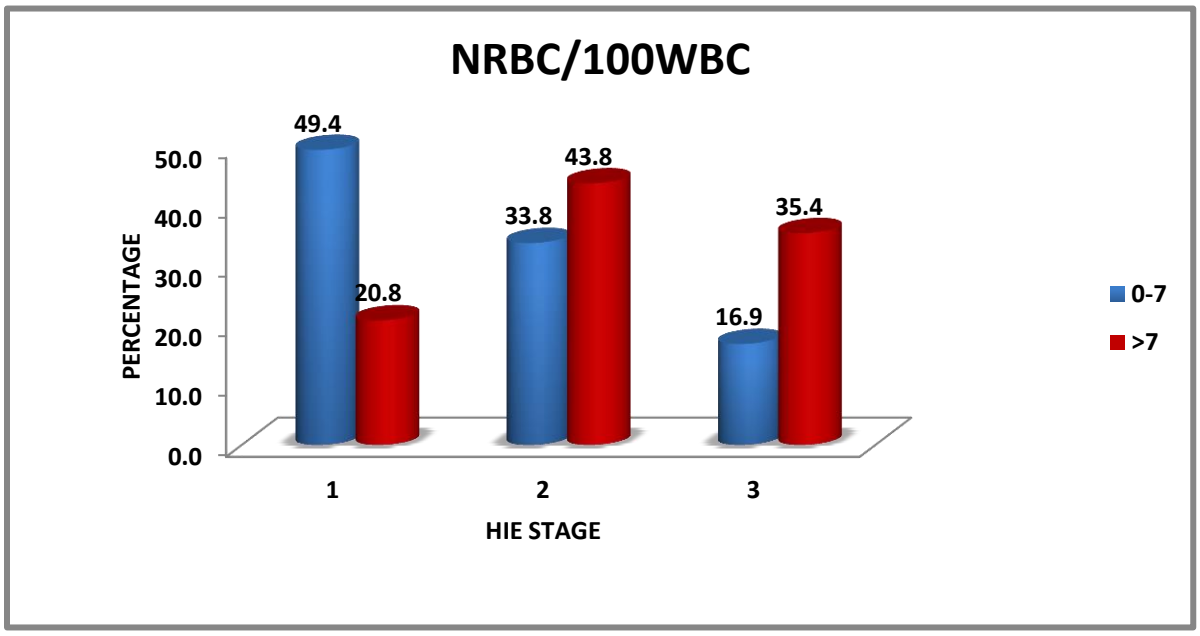
3. Distribution of NRBC/100WBC according to HIE stage

TABLE 19: distribution of NRBC/100WBC according to HIE stage

HIE STAGE	NRBC/100WBC				p value
	0-10		>10		
	N	%	N	%	
1	38	49.4	10	20.8	0.004*
2	26	33.8	21	43.8	
3	13	16.9	17	35.4	
Total	77	100.0	48	100.0	

Note: * significant at 5% level of significance (p<0.05)

FIGURE 19: distribution of NRBC/100WBC according to HIE stage



The two study groups were compared between cases and controls in terms of distribution of Nucleated RBC count according to HIE stage. Table no 19 shows that distribution of Nucleated RBC count and HIE staging have statistical significance that is More is the Number of nucleated RBCs higher is the HIE staging.

NEUROLOGICAL STATUS

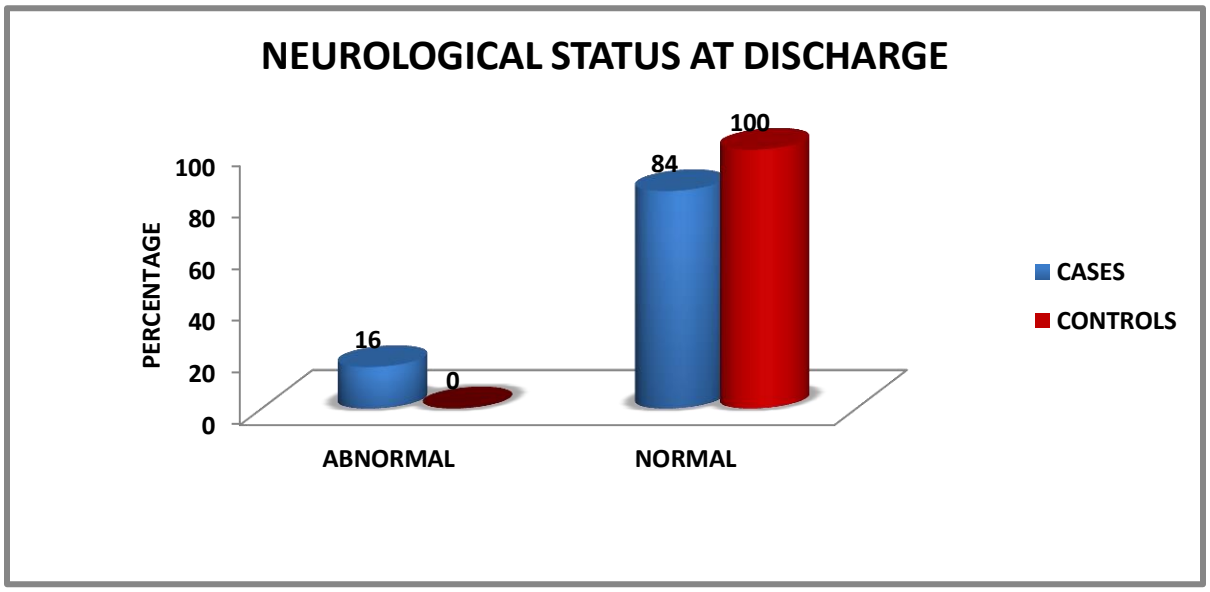
1. **Distribution of neurological status at discharge among cases**

TABLE 20: distribution of neurological status at discharge among cases

NEUROLOGICAL STATUS AT DISCHARGE	CASES		CONTROLS		p value
	N	%	N	%	
ABNORMAL	20	16	0	0	<0.001*
NORMAL	105	84	125	100	
Total	125	100	125	100	

Note: * significant at 5% level of significance (p<0.05)

FIGURE 20: distribution of neurological status at discharge among cases



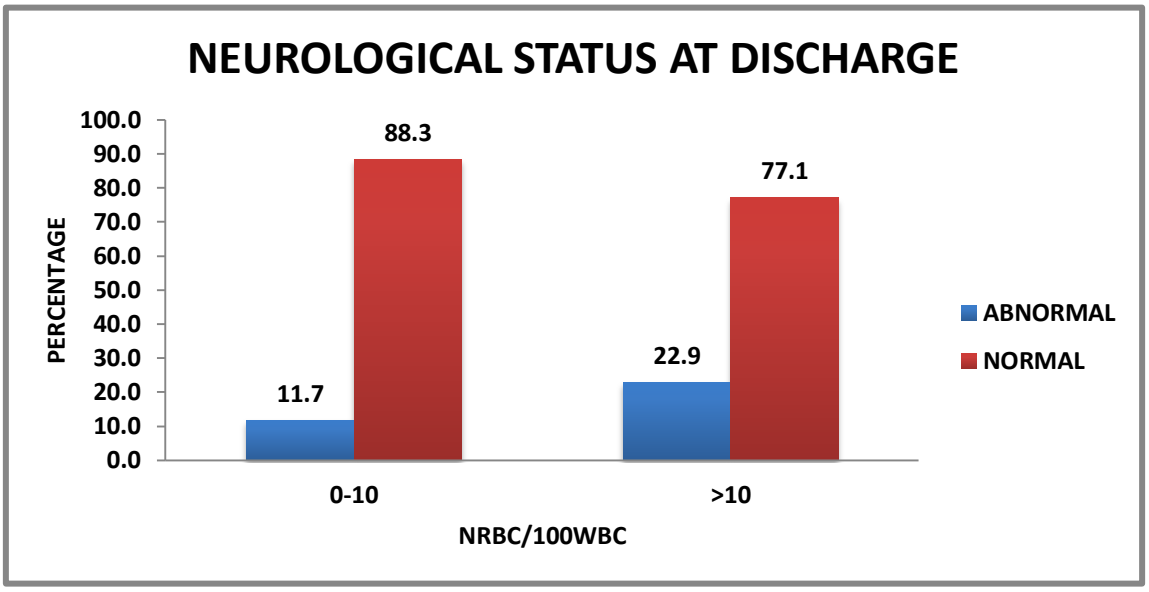
In the above table we can see the Neurological status at discharge of babies among cases and controls and we can see that, the number of abnormally neurological babies are among cases and it is statistically significant.

2. **Association of neurological status at discharge and NRBC/100WBC**

TABLE 21: Association of Neurological Status at discharge and NRBC/100WBC

NEUROLOGICAL STATUS AT DISCHARGE	NRBC/100WBC				p value
	0-10		>10		
	N	%	N	%	
ABNORMAL	9	11.7	11	22.9	0.096
NORMAL	68	88.3	37	77.1	
Total	77	100.0	48	100.0	

FIGURE 21: Association of Neurological Status at discharge and NRBC/100WBC



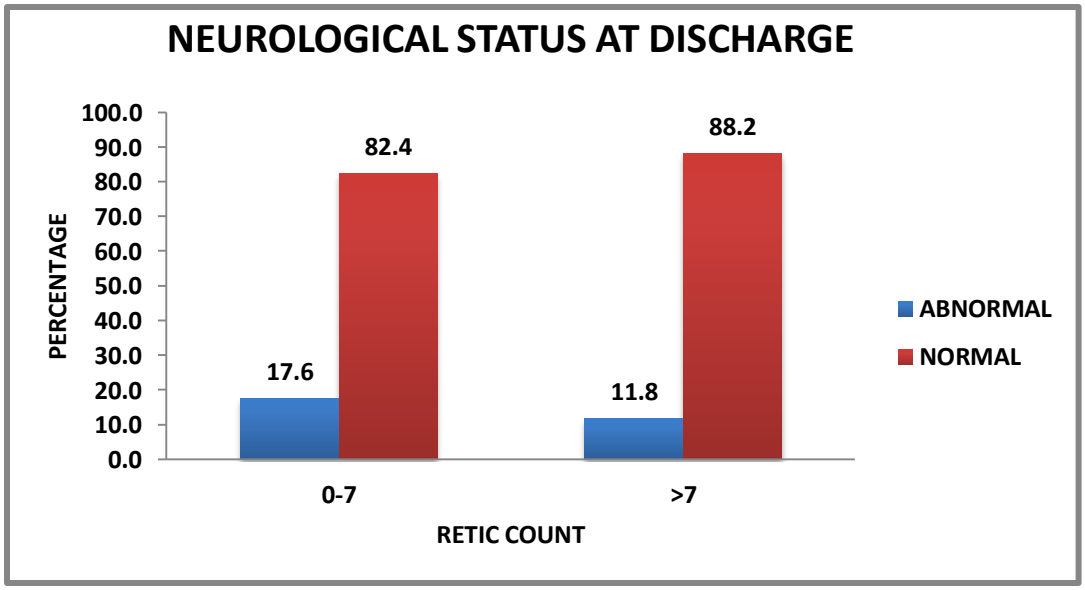
In the above table it shows the association of Neurological status at discharge of babies with Nucleated RBC and we can see that Higher the number of Nucleated RBC, higher is the number of abnormally neurological cases and it is statistically not significant.

3. Association of neurological status at discharge and retic count

TABLE 22: Association of Neurological Status at discharge and retic count

NEUROLOGICAL STATUS AT DISCHARGE	RETIC COUNT				p value
	0-7		>7		
	N	%	N	%	
ABNORMAL	16	17.6	4	11.8	0.43
NORMAL	75	82.4	30	88.2	
Total	91	100.0	34	100.0	

FIGURE 22: Association of Neurological Status at discharge and retic count



In the above table it shows the association of Neurological status at discharge of babies with Reticulocyte counts and it is statistically not significant.

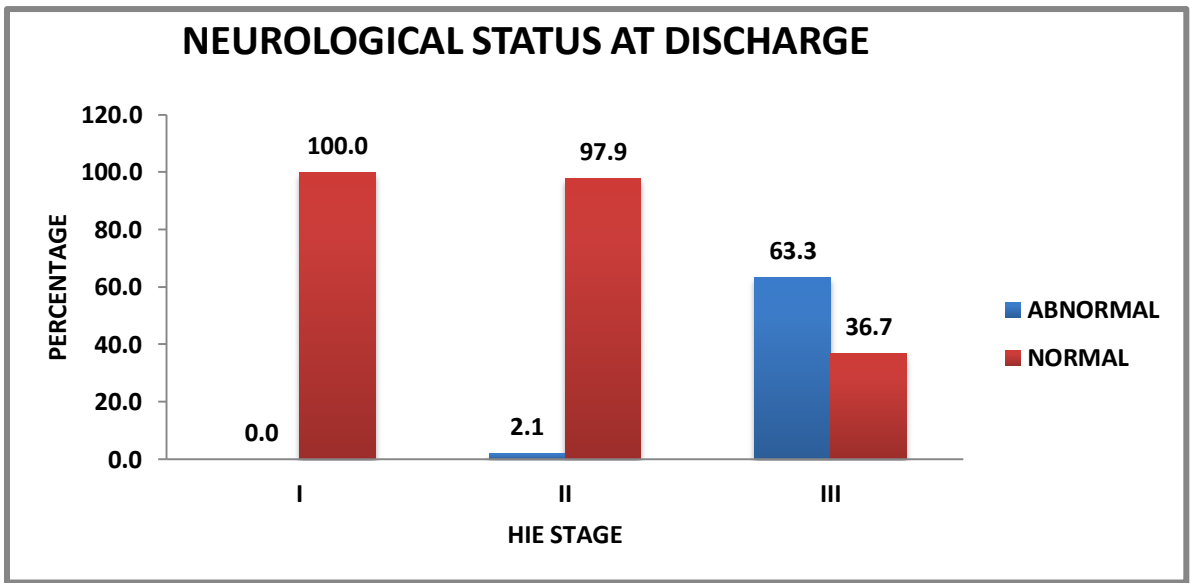
4. **Association of neurological status at discharge and HIE stage**

TABLE 23: Association of Neurological Status at discharge and HIE stage

NEUROLOGICAL STATUS AT DISCHARGE	HIE STAGE						p value
	I		II		III		
	N	%	N	%	N	%	
ABNORMAL	0	0.0	1	2.1	19	63.3	<0.001 *
NORMAL	48	100.0	46	97.9	11	36.7	
Total	48	100.0	47	100.0	30	100.0	

Note: * significant at 5% level of significance (p<0.05)

FIGURE 23: Association of Neurological Status at discharge and HIE stage



In the above table it shows the association of Neurological status at discharge of babies with HIE staging and we can see that Higher the staging, higher is the number of abnormally neurological cases and it is statistically significant.

DURATION OF NICU STAY

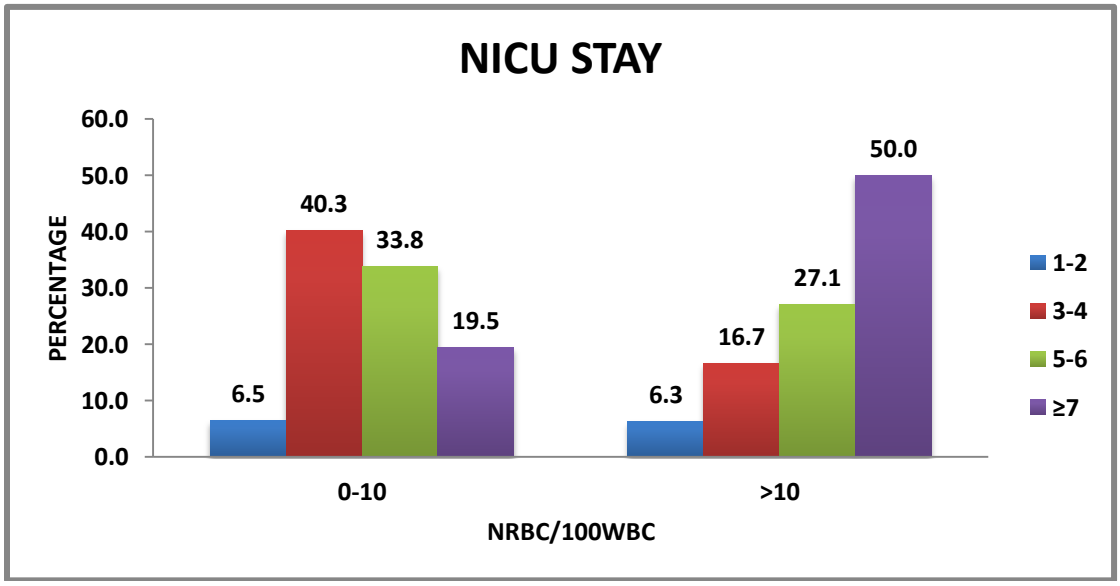
1. Association of NICU stay and NRBC/100WBC

TABLE 24: Association of NICU stay and NRBC/100WBC

NICU STAY	NRBC/100WBC				p value
	0-10		>10		
	N	%	N	%	
1-2	5	6.5	3	6.3	0.002*
3-4	31	40.3	8	16.7	
5-6	26	33.8	13	27.1	
≥7	15	19.5	24	50.0	
Total	77	100.0	48	100.0	

Note: * significant at 5% level of significance (p<0.05)

FIGURE24: Association of NICU stay and NRBC/100WBC



In the above table it shows the association of Duration of NICU stay with Nucleated RBC count and we can see that Higher the number of nRBC, higher is the duration of NICU stay and it is statistically significant.

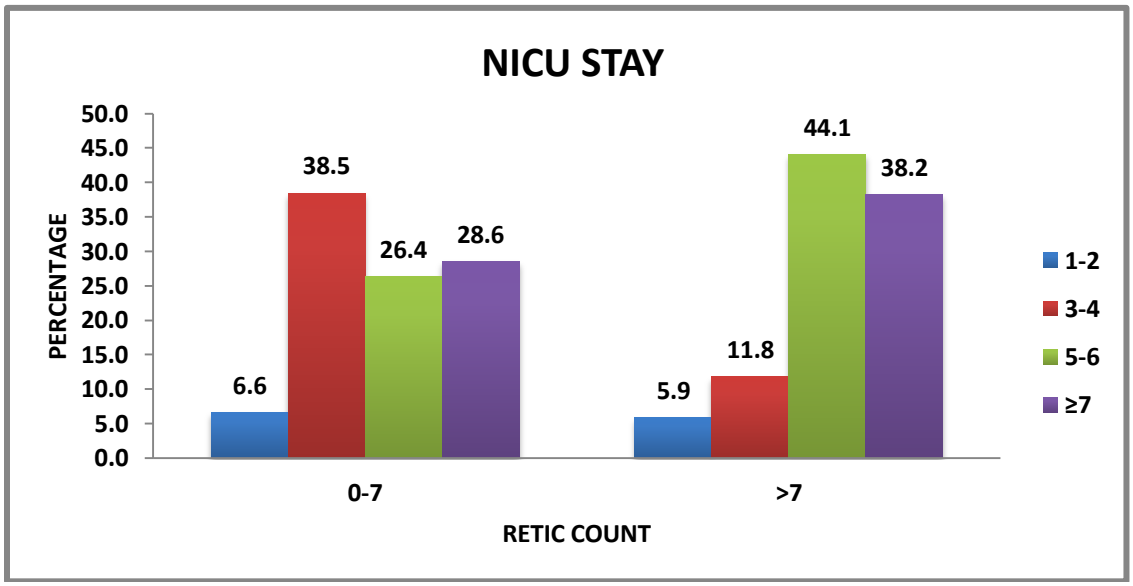
2. Association of NICU stay and retic count

TABLE 25: Association of NICU stay and retic count

NICU STAY	RETIC COUNT				p value
	0-7		>7		
	N	%	N	%	
1-2	6	6.6	2	5.9	0.030*
3-4	35	38.5	4	11.8	
5-6	24	26.4	15	44.1	
≥7	26	28.6	13	38.2	
Total	91	100.0	34	100.0	

Note: * significant at 5% level of significance (p<0.05)

FIGURE 25: Association of NICU stay and retic count



In the above table it shows the association of Duration of NICU stay with Reticulocyte count and we can see that Higher the number of Reticulocytes, higher is the duration of NICU stay and it is statistically significant.

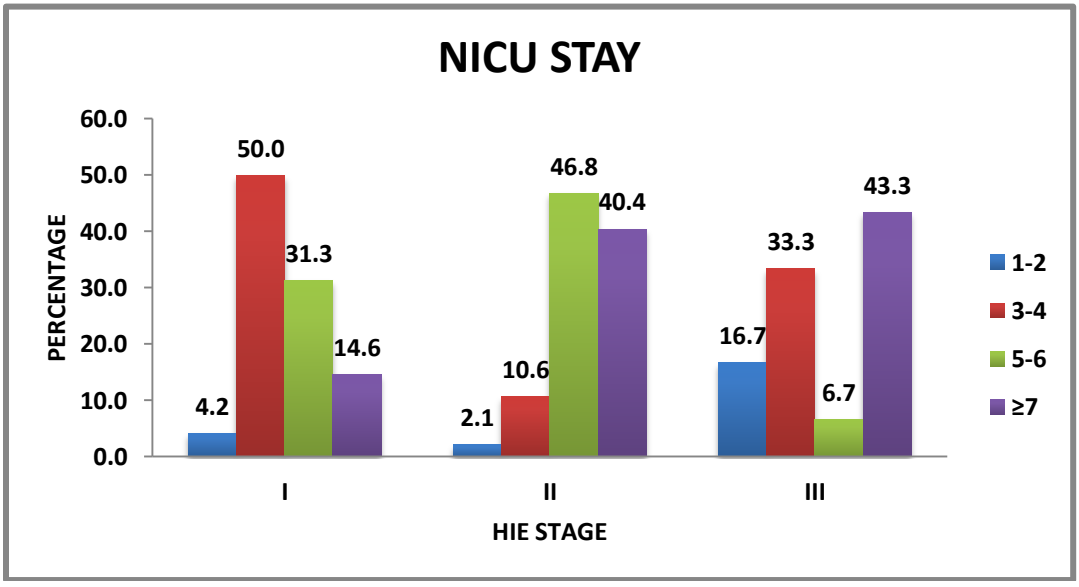
Association of NICU stay and HIE stage

TABLE 26: Association of NICU stay and HIE stage

NICU STAY	HIE STAGE						p value
	I		II		III		
	N	%	N	%	N	%	
1-2	2	4.2	1	2.1	5	16.7	<0.001*
3-4	24	50.0	5	10.6	10	33.3	
5-6	15	31.3	22	46.8	2	6.7	
≥7	7	14.6	19	40.4	13	43.3	
Total	48	100.0	47	100.0	30	100.0	

Note: * significant at 5% level of significance (p<0.05)

FIGURE 26: Association of NICU stay and HIE stage



In the above table it shows the association of Duration of NICU stay with HIE staging and we can see that higher the staging, higher is the duration of NICU stay and it is statistically significant.

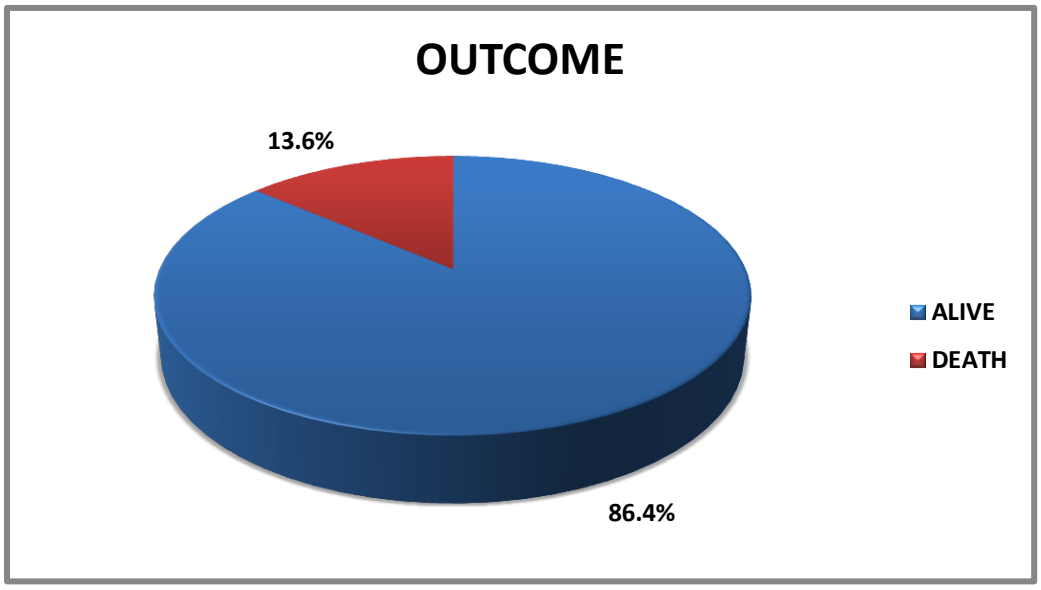
OUTCOME OF THE ASPHYXIATED CASES

1. **Distribution of outcome among cases**

TABLE 27: distribution of outcome among cases

OUTCOME		N	%
ALIVE		108	86.4
DEATH	DAMA	7	5.6
	DEATH	10	8
Total		125	100

FIGURE 27: distribution of outcome among cases



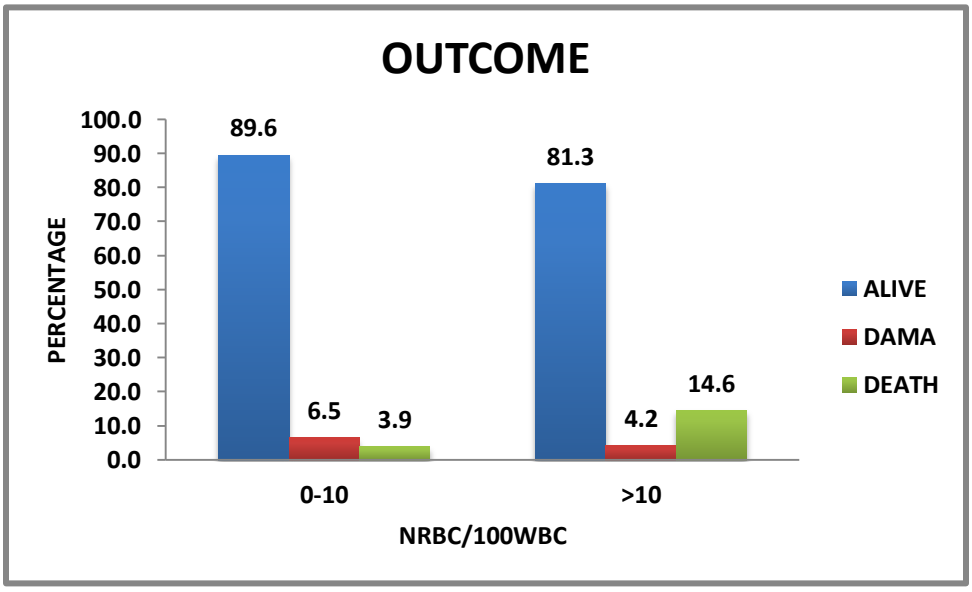
Outcome in cases; out of 125 cases 108 cases that is 86.4% of cases are alive, 7 cases that's 5.6% cases went Discharge Against medical Advice as parents were reluctant to continue treatment for poor prognosis and 10 cases that's 8% of cases died.

2. Association of outcome and NRBC/100WBC

TABLE 28: association of outcome and NRBC/100WBC

OUTCOME	NRBC/100WBC				p value
	0-10		>10		
	N	%	N	%	
ALIVE	69	89.6	39	81.3	0.093
DAMA	5	6.5	2	4.2	
DEATH	3	3.9	7	14.6	
Total	77	100.0	48	100.0	

FIGURE 28: association of outcome and NRBC/100WBC



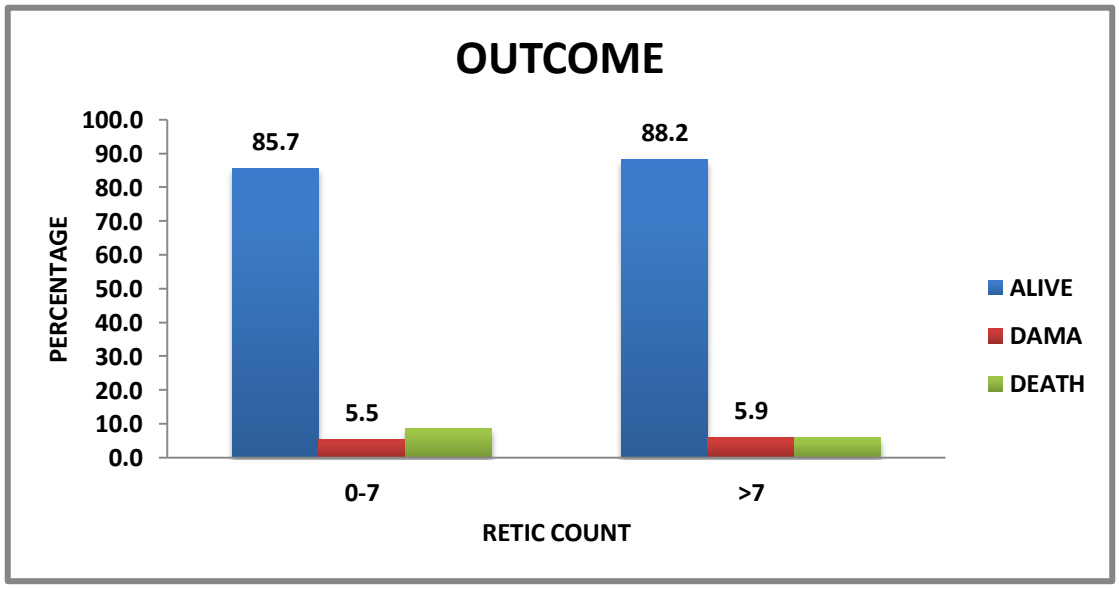
Theabove table compares between association of Outcome of the cases with Nucleated RBC count and we can see that more the number of nucleated RBC higher id the number of death count, but it is statistically not significant.

3. Association of outcome and retic count

TABLE 29: association of outcome and retic count

OUTCOME	RETIC COUNT				p value
	0-7		>7		
	N	%	N	%	
ALIVE	78	85.7	30	88.2	0.866
DAMA	5	5.5	2	5.9	
DEATH	8	8.8	2	5.9	
Total	91	100.0	34	100.0	

FIGURE 29: association of outcome and retic count



Theabove table compares between association of Outcome of the cases with Reticulocyte count and we can see that more the number of Reticulocytes higher is the number of death count, but it is statistically not significant.

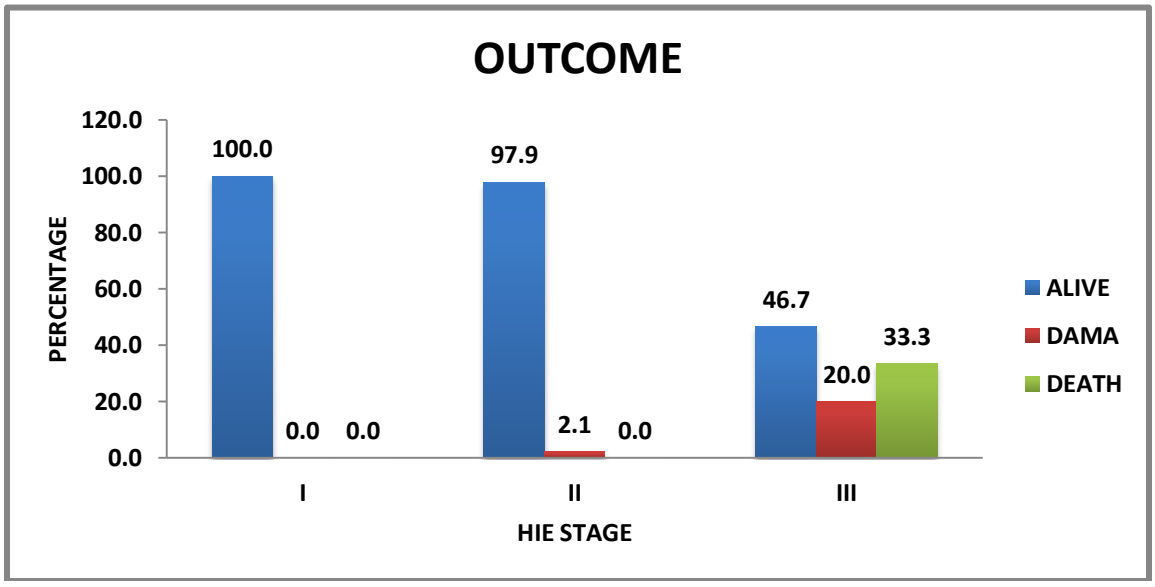
4. Association of outcome and HIE stage

TABLE 30: association of outcome and HIE stage

OUTCOME	HIE STAGE						p value
	I		II		III		
	N	%	N	%	N	%	
ALIVE	48	100.0	46	97.9	14	46.7	<0.001*
DAMA	0	0.0	1	2.1	6	20.0	
DEATH	0	0.0	0	0.0	10	33.3	
Total	48	100.0	47	100.0	30	100.0	

Note: * significant at 5% level of significance (p<0.05)

FIGURE 30: association of outcome and HIE stage



Theabove table compares between association of Outcome of the cases with HIE staging and we can see that higher the staging, higher is the number of death count, but it is statistically significant.

DISCUSSION

DISCUSSION

NRBCs rarely circulate in older children, they are commonly seen in the blood of neonates. They are primarily produced in the fetal bone marrow in response to erythropoietin and are stored in the marrow as precursors to reticulocytes and mature erythrocytes. Many acute and chronic stimuli cause increases in the number of circulating NRBCs from either increased erythropoietic activity or a sudden release from the marrow storage pools. Previously reported causes of a high NRBC count include: prematurity, ABO or Rh incapability, maternal diabetes, intrauterine growth retardation, neonatal sepsis, congenital infection, cyanotic heart disease, pre-eclampsia, maternal smoking, and chorioamnionitis.

However, neonates with these conditions were excluded from the current study. In the present study there were 125 babies in both study groups.

1. There was no significant difference in the sex distribution of babies between the two study groups.
2. The two study groups were similar in terms of parity of mother. But the degree of association of distribution of mothers age between cases and control is statistically significant($p<0.001$).
3. The degree of association of distribution of SES between cases and control is statistically significant($p<0.001$).
4. Otherfactors like Consanguinity, Gestational age previous pregnancy complications like abortions, Asphyxia, Previous death of baby, Past miscarriages have statistical significance($p<0.001$).

5. 37.6% of babies with asphyxia were born in our hospital and 62.4% of babies with asphyxia were referred from other hospitals.
6. 19.2% of babies with asphyxia were Mechanically ventilated, 7.2% of babies with asphyxia needed CPAP support and 88.8% of babies with asphyxia were managed with Oxygen support.
7. 38.4% of babies with asphyxia belonged to HIE stage 1, 37.6% of babies with asphyxia belonged to HIE stage and 24% of babies with asphyxia belonged to HIE stage 3, according to Sarnat & Sarnat staging of asphyxiated babies.
8. Among Hematological parameters; Total Count, C-reactive Protein, NRBC/100 WBCs and Retic Counts have statistical significance, while Differential counts, Hemoglobin, RBC and Platelet counts have no statistical significance.
9. In our study mean APGAR scores were 7 at 1^{st} min, 8.5 at 5^{th} min and 9 at 10^{th} min in controls whereas 4.7 at 1^{st} min, 6.4 at 5^{th} min and 6.9 at 10^{th} min in cases which have statistical significance($p<0.001$).

Reticulocyte counts

1. The mean reticulocyte count among cases is 5.9% and controls is 4.2% and can conclude that the mean reticulocyte count is more and is statistically significant($p<0.001$).
2. Our study also shows that Reticulocyte counts of 0-7% were more in controls whereas >7% were more in cases which have statistical significance($p<0.001$). The distribution of reticulocyte count and HIE staging have No statistical significance.

Nucleated RBC count in normal new-born

Nucleated red blood cells are commonly seen in the cord blood of healthy new-borns at birth. In term non-asphyxiated new-borns, the number of nucleated red blood cells is variable but is only rarely higher than 10/100WBC. The nucleated RBC count in normal new-borns in various previous studies is shown in Table 2.

REFERENCE	SAMPLE SIZE	NRBC	AGE	GESTATION/ BIRTH WEIGHT
Naeye(61)	84	919±1425 nrbc/mm^3	1 hour	term
Green(49)	102	400±1300 nrbc/mm^3	12-24 hours	37-41 weeks AGA
Sinha(241)	84	2.3 ± 0.7 NRBC/100WBCs	Birth (Cord blood)	2501-3500 grams
Shivhare(242)	33	4.1 ± 2.4 NRBC/100WBCs	Birth (Cord blood)	Term and near term
Phelan(234)	83	3.4 ± 3.0 NRBC/100WBCs	Birth (Cord blood)	≥ 37 weeks, > 2700 gms
Hanlon-lundberg(244)	1112	8.5 ± 10.3 NRBC/100WBCs	Birth (Cord blood)	37 – 41 weeks*
Buonocore(15)	105	1689 ± 290 NRBC/100 WBCs	Birth (Cord blood)	37 – 41 weeks*
Axt(245)	304	3.7 (median) NRBC/100 WBCs	Birth (Cord blood)	261 – 289 days
		6.5 (median) NRBC/100 WBCs	Birth (Cord blood)	290 + days

1. In our study the mean nRBC count among cases is 15.3 and controls is 2.0 and can conclude that the mean nRBC count is more and is statistically significant($p<0.001$).
2. Our study also shows that nucleated RBC counts of 0-10 were more in controls whereas >10 were more in cases which have statistical significance($p<0.001$).
3. The distribution of Nucleated RBC count and HIE staging have statistical significance that is More is the Number of nucleated RBCs higher is the HIE staging($p<0.001$).

Neurological status

1. The association of Neurological status at discharge of babies with Nucleated RBC and we can see that Higher the number of Nucleated RBC, higher is the number of abnormally neurological cases and but it is statistically not significant.
2. The association of Neurological status at discharge of babies with Reticulocyte counts and it is statistically not significant.
3. The association of Neurological status at discharge of babies with HIE staging and we can see that Higher the staging, higher is the number of abnormally neurological cases and it is statistically significant($p<0.001$).

Duration of NICU stay

1. The association of Duration of NICU stay with Nucleated RBC count and we can see that Higher the number of nRBC, higher is the duration of NICU stay and it is statistically significant($p<0.001$).
2. The association of Duration of NICU stay with Reticulocyte count and we can see that Higher the number of Reticulocytes, higher is the duration of NICU stay and it is statistically significant($p<0.001$).
3. The association of Duration of NICU stay with HIE staging and we can see that higher the staging, higher is the duration of NICU stay and it is statistically significant($p<0.001$).

Outcome

1. Out of 125 cases 108 cases that is 86.4% of cases are alive, 7 cases that's 5.6% cases went Discharge Against medical Advice as parents were reluctant to continue treatment for poor prognosis and 10 cases that's 8% of cases died.
2. The association of Outcome of the cases with Nucleated RBC count and we can see that more the number of nucleated RBC higher id the number of death count, but it is statistically not significant.
3. The association of Outcome of the cases with Reticulocyte count and we can see that more the number of Reticulocytes higher is the number of death count, but it is statistically not significant.
4. The association of Outcome of the cases with HIE staging and we can see that higher the staging, higher is the number of death count, but it is statistically significant.

Limitations of our study:

1. Study involved only term new-borns. Therefore, it cannot be generalized to whole neonatal population.
2. Study followed babies only up to discharge. Therefore, the association of nucleated RBC count with long term neurological outcome can just be corelated with short term outcome.
3. This study did not correlate NRBC count and reticulocyte counts with pH which is reported to be a reliable marker of perinatal asphyxia.
4. This study involved in a setup where more than half of the cases were referred from outside delivered hospitals, in which history is not reliable.

CONCLUSIONS

- Nucleated red blood cell count has significant positive correlation withseverity of hypoxic ischemic encephalopathy
- Nucleated red blood cell counthas significant positive correlation withneurological impairmentfollowing birth asphyxia.
- Nucleated red blood cell count has significant positive correlation withduration of NICU stay.
- Reticulocyte count has significant positive correlation with severity of hypoxic ischemic encephalopathy
- Reticulocyte count has significant positive correlation with neurological impairment following birth asphyxia.
- Reticulocyte count has significant positive correlation with duration of NICU stay.
- Severity of hypoxic ischemic encephalopathy has significant positive correlation with duration of NICU stay.
- Neurological impairment following birth asphyxia has significant positive correlation with severity of hypoxic ischemic encephalopathy.
- Outcome of the Asphyxiated baby is not statistically significant with nucleated red blood cell count and Reticulocyte count
- Outcome of the Asphyxiated baby has significant positive correlation with severity of hypoxic ischemic encephalopathy
- Nucleated red blood cell count can be used as surrogate marker for birth asphyxia.

Nucleated RBC count and reticulocyte counts in peripheral venous blood or cord blood is a low cost, simple and easily available test which can be done in any health care facility with minimal infrastructure. In our country, a large number of deliveries occur in peripheral health facilities and at home by trained and untrained birth attendants. In such cases, accurate and reliable recording of well-established markers of birth asphyxia like Apgarscores, intranataltocography recording for fetal distress, fetal scalp pH monitoring is often not available. Such babies pose diagnostic dilemmas for treating doctors in tertiary health care centres where these babies are referred. Early and accurate diagnosis of birth asphyxia is crucial in determining both short term and long-term prognosis. Cord blood NRBC count has been established as a marker of perinatal asphyxia in many previous studies. The present study establishes the role of NRBC count as well as Reticulocyte count in peripheral venous blood collected within 6 hours of birth in diagnosis and prognostication of birth asphyxia. Hence, this simple and reliable test can routinely be included in investigation of all new-borns with suspected birth asphyxia for diagnosis as well as prognosis.

SUMMARY

SUMMARY

A one and half year Prospective Observational Comparative study was done to know relation between peripheral venous blood nucleated red cell count collected within 6 hours of birth and perinatal asphyxia was carried out in the Department of Paediatrics,Shri.B.M.Patil Medical College, Hospital & Research Centre, Vijayapur. Total number of babies enrolled was 250 (125 cases and 125 controls). In the present study there were 125 babies in both study groups. There was no significant difference in the sex distribution of babies between the two study groups. There was no significant difference in the sex distribution of babies between the two study groups. The two study groups were similar in terms of parity of mother. But the degree of association of distribution of mothers age between cases and control is statistically significant($p<0.001$). The degree of association of distribution of SES between cases and control is statistically significant($p<0.001$). Other factors like Consanguinity, Gestational age previous pregnancy complications like abortions, Asphyxia, Previous death of baby, Past miscarriages have statistical significance($p<0.001$).37.6% of babies with asphyxia were born in our hospital and 62.4% of babies with asphyxia were referred from other hospitals. 19.2% of babies with asphyxia were Mechanically ventilated, 7.2% of babies with asphyxia needed CPAP support and 88.8% of babies with asphyxia were managed with Oxygen support.38.4% of babies with asphyxia belonged to HIE stage 1, 37.6% of babies with asphyxia belonged to HIE stage and 24% of babies with asphyxia belonged to HIE stage 3, according to Sarnat & Sarnat staging of asphyxiated babies.Among Hematological parameters; Total Count, C-reactive Protein, NRBC/100 WBCs and Retic Counts have statistical significance, while Differential counts, Hemoglobin, RBC and

Platelet counts have no statistical significance.In our study mean APGAR scores were 7 at 1st min, 8.5 at 5th min and 9 at 10th min in controls whereas 4.7 at 1st min, 6.4 at 5th min and 6.9 at 10th min in cases which have statistical significance($p<0.001$). The association of Neurological status at discharge of babies with Nucleated RBC and we can see that Higher the number of Nucleated RBC, higher is the number of abnormally neurological cases and but it is statistically not significant. The association of Neurological status at discharge of babies with Reticulocyte counts and it is statistically not significant. The association of Neurological status at discharge of babies with HIE staging and we can see that Higher the staging, higher is the number of abnormally neurological cases and it is statistically significant($p<0.001$). The association of Duration of NICU stay with Nucleated RBC count and we can see that Higher the number of nRBC, higher is the duration of NICU stay and it is statistically significant($p<0.001$). The association of Duration of NICU stay with Reticulocyte count and we can see that Higher the number of Reticulocytes, higher is the duration of NICU stay and it is statistically significant($p<0.001$). The association of Duration of NICU stay with HIE staging and we can see that higher the staging, higher is the duration of NICU stay and it is statistically significant($p<0.001$). Out of 125 cases 108 cases that is 86.4% of cases are alive, 7 cases that's 5.6% cases went Discharge Against medical Advice as parents were reluctant to continue treatment for poor prognosis and 10 cases that's 8% of cases died. The association of Outcome of the cases with Nucleated RBC count and we can see that more the number of nucleated RBC higher id the number of death count, but it is statistically not significant.

The association of Outcome of the cases with Reticulocyte count and we can see that more the number of Reticulocytes higher is the number of death count, but it is statistically not significant.The association of Outcome of the cases with HIE staging and we can see that higher the staging, higher is the number of death count, but it is statistically significant.

REFERENCES

REFERENCES

1. World Health Organization. Neonatal and Perinatal Mortality; Country, Regional and Global estimates,2004. Geneva: WHO, 2006. 1-25.

2. Committee on foetus and new born, American Academy of Paediatrics and Committee on obstetric practice, American College of Obstetrics and Gynaecology. Use and abuse of the APGAR score. Paediatrics 1996; 98(1): 141-42.

3. NNPD Network. Neonatal Neonatal-Perinatal Database, Report 2002–2003. Deorari A, ed. New Delhi: Indian Council of Medical Research; 2005.

4. World Health Organization. International Statistical Classification of Diseases and Related Health Problems, Tenth Revision (ICD-10). Chapter XVI: Certain conditions originating in the perinatal period (P00-P96). Geneva: World Health Organization. 1992.

5. Goldstein P. "Birth asphyxia". In Historical review and recent advances in neonatal and perinatal medicine. G. Smith and D. Vidyasagar (Ed.). Illinois: Mead Johnson Nutritional Division;1980. Vol.1:177-184.

6. Lippman HS. Morphologic and quantitative study of blood corpuscles in the new born period. Am J Dis Child 1924; 27: 473-526.

7. Ryerson CS, Sanes S. The age of pregnancy. Histologic diagnosis from percentageof erythroblasts in chorionic capillaries. Arch Pathol 1934; 17: 648–51.

8. Javert CT. The occurrence and significance of nucleated erythrocytes in the fetalvessels of the placenta. Am J ObstetGynecol 1939; 37: 184-94.

9. Anderson GW. Studies on the nucleated red blood cell count in the chorioniccapillaries and the cord blood of various ages of pregnancy. Am J Obstet Gynecol1941; 42: 1-14.

10. Fox H. The incidence and significance of nucleated erythrocytes in the fetalvessels of the mature human placenta. J ObstetGynaecol Br Commonw 1967; 74: 40-3

11. Ghosh B, Mittal S, Kumar S, Dadhwal V. Prediction of perinatal asphyxia with nucleated red blood cells in cord blood of newborns. Int J GynaecolObstet 2003; 81(3): 267-71.77

12. Ferber A, Grassi A, Akyol D, O'Reilly-Green C, Divon MY. The association of fetal heart rate patterns with nucleated red blood cell counts at birth. Am J ObstetGynecol 2003; 188 (5) 1228-30.

13. Mansanoc RZ, Frasch M, McPhaul L, Gagnon R, Richardson B, Ross M. Ovine nucleated red blood cell count as a marker for antepartum asphyxia. Am J ObstetGynecol 2007; 197 (6): S151.

14. Hanlon-Lundberg KM, Kirby RS. Nucleated red blood cells as a marker of acidemia in term neonates, Am J ObstetGynecol 1999; 181 (1): 196-201.

15. Buonocore G, Perrone S, Gioia D, Gatti MG, Massafra C, Agosta R et al. Nucleated red blood cell count at birth as an index of perinatal brain damage. Am J ObstetGynecol 1999; 181(6): 1500-5.

16. Feber A, Akyol D, Kane LA, Grassi A, Divon MY. Nucleated red blood cells in human fetal scalp capillary blood samples: A feasibility study. J Matern Fetal Neonatal Med 2002; 11: 26-9.

17. Manroe BL, Weinberg AG, Rosenfeld CR, Browne R. The neonatal blood count in health and disease: Reference values for neutrophilic cells. J Pediatr 1979; 95: 89-98.

18. Bax MC, Flodmark O, Tydeman C. Definition and classification of cerebral palsy. From syndrome toward disease. Dev Med Child Neurol Suppl. 2007;109:39-41.

19. Volpe JJ. Neurology of the newborn. 4th edition. Philadelphia: WB Saunders Company, 2001, pp. 217-76.

20. Lorek A, Takei Y, Cady EB, Wyatt JS, Penrice J, Edwards AD, Peebles D, Wylezinska M, Owen-Reece H, Kirkbride V, Cooper CE, Aldridge RF, Roth SC, Brown G, Delpy DT, Reynolds EOR. Delayed ("secondary") cerebral energy failure after acute hypoxia-ischemia in the newborn piglet: continuous 48-hour studies by phosphorus magnetic resonance spectroscopy. Pediatr Res. 1994;36(6):699-706.

21. Johnston MV, Trescher WH, Ishida A, Nakajima W. Neurobiology of hypoxic-ischemic injury in the developing brain. Pediatr Res. 2001;49(6):735-41.

22. Siesjö BK, Bengtsson F. Calcium fluxes, calcium antagonists, and calcium-related pathology in brain ischemia, hypoglycemia, and spreading depression: a unifying hypothesis. J Cereb Blood Flow Metab. 1989;9:127-40.

23. Johnson MV et, al; Neurobiology of hypoxic-ischemic injury in the developing brain, pediatr res 49:735,2001

24. Lapointe A, Barrington KJ. Pulmonary hypertension and the asphyxiated newborn. J Pediatr. 2011;158(2 Suppl):e19-24.

25. Phelan JP, Korst LM, Martin GI. Application of criteria developed by the Task Force on Neonatal Encephalopathy and Cerebral Palsy to acutely asphyxiated neonates. Obstet Gynecol. 2011;118:824-30.

26. Hankins GD, Koen S, Gei AF, Lopez SM, Van Hook JW, Anderson GD. Neonatal organ system injury in acute birth asphyxia sufficient to result in neonatal encephalopathy. Obstet Gynecol. 2002;99(5 Pt 1):688-91.

27. Efron D, South M, Volpe JJ, Inder T. Cerebral injury in association with profound iatrogenic hyperglycemia in a neonate. Eur J Paediatr Neurol. 2003;7(4):167-71.

28. Finch CA, Harker LA, Cook JD. Kinetics of the formed elementsof human blood. *Blood 1977;50:699-707.*

29. McMullin MF, Lappin TRJ, Elder GE, Savage GA, Bridges JM. Erythropoieticresponse to hypobaric hypoxia in rabbits. Biomed BiochemActa 1988; 47: 523-7.

30. Eckardt KU, Boutellier U, Kurtz A, Schopen M, Koller EA, Bauer C. Rate of erythropoietin formation in humans in response to acute hypobaric hypoxia. J Appl Physiol 1989; 66: 1785–8.

31. Maier RF, Bohme K, Dudenhausen JW, Obladen M. Cord blood erythropoietin in relation to different markers of fetal hypoxia. ObstetGynecol 1993; 81(4):

32. Widness JA, Sawyer ST, Schmidt RL, Chestnut DH. Lack of maternal to fetal transfer of I labelled erythropoietin in sheep. J Dev Physiol 1991; 15: 139-43.

33. Cole RJ, Paul J. The effects of erythropoietin on heam synthesis in mouse yolk sac and cultured fetal cells. J Embryol Exp Morphol 1966; 15: 245- 60.

34. Paul J, Conkie D, Freshney RI. Erythropoietic cell population changes during the hepatic phase of erythropoiesis in the fetal mouse. Cell Tissue Kinet 1969; 2: 283-94.

35. Rich IN, Kubanek B. The ontogeny of erythropoiesis in the mouse detected by erythroid colony forming technique. J Embryol Exp Morphol 1980; 58: 143-55.

36. Forestier F, Daffos F, Catherine N, Renard M, Andreux JP. Developmental hematopoiesis in normal human fetal blood. Blood 1991; 77: 2360–3.

37. Erslev AJ, Beutler E. Action of erythropoietin. In: Beutler E, Lichtman MA, Coller BS, *et al*, eds. Williams hematology. 5th ed. New York: McGraw-Hill 1995:435–6.

38. Iversen PO, Nicolaysen G, Benestal HB. Blood flow to bone marrow during development of anemia or polycythemia in the rat. Blood 1992; 79 (3): 594-601.

39. Iversen PO. Blood flow to the haematopoietic bone marrow. Acta PhysiolScand 1997; 159 (4): 269-76.

40. John P, Foerster J, Lukens JN, Rodgers GM, Paraskevas F, Gladder B et al. Wintrobe's clinical hematology. 11th Ed. Philadelphia: Lippincotts Williams and Wilkins; 2003.

41. Sarnat HB, Sarnat MS. Neonatal encephalopathy following fetal distress. A clinical and electroencephalographic study. Arch Neurol. 1976;33:696–705.

42. Maier RF, Bohme K, Dudenhausen JW, Obladen M. Cord blood erythropoietin in relation to different markers of fetal hypoxia. ObstetGynecol 1993; 81(4): 575–80.

43. Baschat AA, Gungor S, Kush ML, Berg C, Gembruch U, Harman CR. Nucleated red blood cell counts in the first week of life: a critical appraisal of relationships with perinatal outcome in preterm growth- restricted neonates. Am J ObstetGynecol 2007; 197 (3): 286.e1-8.

44. Varvarigou A, Beratis NG, Makri M, Vagenakis AG. Increased levels and positive correlation between erythropoietin and hemoglobin concentrations in newborn children of mothers who are smokers. J Pediatr 1994; 124: 480-2. 82

45. Mamopoulos M, Bill H, Tsantali C, Assimakopoulos E, Mantalenakis S, Farmakides G. Erythropoietin umbilical serum levels during labor in women with preeclampsia, diabetes, and preterm labor. Am J Perinatol 1994; 11 (6):427-9.

46. Kathleen M, Hanlon-Lundberg, Kirby RS. Association of ABO incompatibility with elevation of nucleated red blood cell counts in term neonates. Am J ObstetGynecol 2000; 183 (6): 1532-6.

47. Leikin E, Verma U, Klein S, Tejani N. Relationship between neonatal nucleated red blood cell counts and hypoxic ischemic injury. ObstetGynecol 1996; 87: 439-43.

48. Dollberg S, Fainaru O, Mimouni FB, Shenhav M, Lessing JB, Kupferminc M. Effect of passive smoking in pregnancy on neonatal nucleated red blood cells. Pediatrics 2000; 106(3): E34.

49. Green DW, Mimouni G. Nucleated erythrocytes in healthy infants and in infants of diabetic mothers. J Pediatr 1990; 166: 129–31

50. Hanlon-Lundberg KM, Kirby RS, Gandhi S, Broekhuizen FF, Niebyl JR. Nucleated red blood cells in cord blood of singleton term neonates. Am J ObstetGynecol 1997; 176: 1149-56.

51. Yeruchimovich M, Mimouni FB, Green DW, Dollberg S. Nucleated red blood cells in healthy infants of women with gestational diabetes. ObstetGynecol 2000:95:84–6.

52. Salvesen DR, Brudenell JM, Snijders RJM, Ireland RM, Nicolaides KH. Fetal plasma erythropoietin in pregnancies complicated by maternal diabetes mellitus. Am J ObstetGynecol 1993; 168: 88-94.

53. Oski FA, Naiman JL. Normal blood values in the newborn period. In: Hematologic problems in the newborn. 2nd Ed. Philadelphia: WB Saunders, 1972:1-30.

54. Ingall D, Sanchez PJ. Syphilis. In: Remington JS, Klein JO, eds. Infectious diseases of the fetus and newborn infant. 5th ed. Philadelphia: WB Saunders, 2001:654.

55. Boskabadi H, Maamouri GH, Sadeghian MH, Ghayour-Mobarhan M, Heidarzade M, Shakeri MT. early diagnosis of perinatal asphyxia by nucleated red blood cell count: A case control study. Arch Iran Med 2010; 13(4): 275-81.

56. Blackwell SC, Refuerzo JS, Wolfe HM, Hassan SS, Berry SM, Sokol RJ et al. The relationship between nucleated red blood cell counts and early-onset neonatal seizures. Am J ObstetGynecol 2000; 182: 1452–7.

57. Thilaganathan B, Athanasious S, Ozmen S, Creighton S, Watson NR, Nicolaides KH. Umbilical cord blood erythroblast count as an index of intrauterine hypoxia. Arch Dis Child Fetal Neonatal Ed 1994; 70(3): F192-4.

58. Merenstein GB, Blackmon LR, Kushner J. Nucleated red-cells in the newborn. Lancet 1970; 1(7659): 1293-4.

59. Korst LM, Phelan JP, Ahn MO, Martin GI. Nucleated red blood cells: An update on the marker for fetal asphyxia. Am J ObstetGynecol 1996; 175:843-6. 84

60. Phelan JP, Korst LM, Young Ock AHN, Martin GI. Neonatal nucleated red blood cell and lymphocyte counts in fetal brain injury. ObstetGynecol 1998; 91: 485-9.

61. Naeye RL, Localio AR. Determining the time before birth when ischemia and hypoxemia initiated cerebral palsy. ObstetGynecol 1995; 86: 713-19.

62. Atshuler G, Hyder SR. Nucleated erythrocytes. In: Pitkin RM, Scott JR, eds. Clinical obstetrics and gynecology. Philadelphia: Lippincott-Raven, 1996:553–6.

63. Benirschke K. Placenta pathology questions to the perinatologist. J Perinatol 1994; 14: 371-5.

64. Fanaroff AA. In: Fanaroff AA, Maisels MJ, Stevenson DK, eds. Year Book of Neonatal and Perinatal Medicine 1997. St. Louis: Mosby, 1997:331.

65. Naeye RL. How to time when hypoxic-ischemic fetal brain damage took place. In: Maulik D, ed. Asphyxia and fetal brain damage New York: Wiley-Liss 1998:153–8.

66. Maier RF, Gunther A, Vogel M, Dudenhausen JW, Obladen M. Umbilical venous erythropoietin and umbilical arterial pH in relation to morphologic placental abnormalities. ObstetGynecol 1994; 84: 81-7.

67. Salafia CM, Ghidini A, Pezzullo JC, Rosenkrantz TS. Early neonatal nucleated erythrocyte counts in preterm deliveries: Clinical and pathologic correlations. J Soc GynecolInvestig 1997; 4 (3): 138-43.

68. Leiken E, Garry D, Visintainer P, Tejani N. Correlation of neonatal nucleated red blood cell counts in preterm infants with histologic chorioamnionitis. Am J ObstetGynecol 1997; 177: 27-30.

69. Miller DR. Neonatal and postnatal erythropoiesis. In: Miller DR, Baehner RL, eds. Blood diseases of infancy and childhood. 7th ed. St Louis: CV Mosby, 1995:153.

70. Phelan JP, Ahn MO, Korst LM, Martin GI. Nucleated red blood cells: a marker for fetal asphyxia? Am J ObstetGynecol 1995; 173: 1380-4.

71. Prechtl HF, Einspieler C, Cioni G, Bos AF, Ferrari F, Sontheimer D. An early marker for neurological deficits after perinatal brain lesions. Lancet. 1997;349:1361–3.

72. Ferrari F, Todeschini A, Guidotti I, Martinez-Biarge M, Roversi MF, Berardi A, et al. General movements in full-term infants with perinatal asphyxia are related to basal ganglia and thalamic lesions. J Pediatr. 2011;158:904–11

73. Herrera-Marschitz M, Morales P, Leyton L, Bustamante D, Klawitter V, Espina-Marchant P, et al. Perinatal asphyxia: current status and approaches towards neuroprotective strategies, with focus on sentinel proteins. Neurotox Res. 2011;19:603–27

74. Northington FJ, Zelaya ME, O'Riordan DP, Blomgren K, Flock DL, Hagberg H, et al. Failure to complete apoptosis following neonatal hypoxia-ischemia manifests as "continuum" phenotype of cell death and occurs with multiple manifestations of mitochondrial dysfunction in rodent forebrain. Neuroscience. 2007;149:822–33.

75. Hagberg H, Mallard C, Rousset CI, Xiaoyang W. Apoptotic mechanisms in the immature brain: involvement of mitochondria. J Child Neurol. 2009;24:1141–6.

76. Ginet V, Puyal J, Clarke PG, Truttmann AC. Enhancement of autophagic flux after neonatal cerebral hypoxia-ischemia and its region-specific relationship to apoptotic mechanisms. Am J Pathol. 2009;175:1962–74.

77. Eisenberg-Lerner A, Bialik S, Simon HU, Kimchi A. Life and death partners: apoptosis, autophagy and the cross-talk between them. Cell Death Differ. 2009;16:966–75.

78. Bonfoco E, Krainc D, Ankarcrona M, Nicotera P, Lipton SA. Apoptosis and necrosis: two distinct events induced, respectively, by mild and intense insults with N-methyl-D-aspartate or nitric oxide/superoxide in cortical cell cultures. Proc Natl Acad Sci USA. 1995;92:7162–6.

79. Yuan J, Yankner BA. Apoptosis in the nervous system. Nature. 2000;407:802–9.

80. Morales P, Fiedler JL, Andres S, Berrios C, Huaiquin P, Bustamante D, et al. Plasticity of hippocampus following perinatal asphyxia: effects on postnatal apoptosis and neurogenesis. J Neurosci Res. 2008;86:2650–62.

81. Dell'Anna E, Chen Y, Loidl F, Andersson K, Luthman J, Goiny M, et al. Short-term effects of perinatal asphyxia studied with Fos-immunocytochemistry and in vivo microdialysis in the rat. Exp Neurol. 1995;131:279–87.

82. Kirino T, Tamura A, Sano K. Selective vulnerability of the hippocampus to ischemiareversible and irreversible types of ischemic cell damage. Prog Brain Res. 1985;63:39–58.

83. Northington FJ, Ferriero DM, Graham EM, Traystman RJ, Martin LJ. Early neurodegeneration after hypoxia-ischemia in neonatal rat is necrosis while delayed neuronal death is apoptosis. Neurobiol Dis. 2001;8:207–19.

84. Ness JM, Harvey CA, Strasser A, Bouillet P, Klocke BJ, Roth KA. Selective involvement of BH3-only Bcl-2 family members Bim and Bad in neonatal hypoxia-ischemia. Brain Res. 2006;1099:150–9.

85. Chen J, Zhu RL, Nakayama M, Kawaguchi K, Jin K, Stetler RA, et al. Expression of the apoptosis-effector gene, Bax, is upregulated in vulnerable hippocampal CA1 neurons following global ischemia. J Neurochem. 1996;67:64–71.

86. Graham EM, Sheldon RA, Flock DL, Ferriero DM, Martin LJ, O'Riordan DP, et al. Neonatal mice lacking functional Fas death receptors are resistant to hypoxic-ischemic brain injury. Neurobiol Dis. 2004;17:89–98.

87. Cheng Y, Black IB, DiCicco-Bloom E. Hippocampal granule neuron production and population size are regulated by levels of bFGF. Eur J Neurosci. 2002;15:3–12.

88. Golan H, Huleihel M. The effect of prenatal hypoxia on brain development: short- and long-term consequences demonstrated in rodent models. Dev Sci. 2006;9:338–49.

89. Ferrer I, Pozas E, Marti M, Blanco R, Planas AM. Methylazoxymethanol acetate-induced apoptosis in the external granule cell layer of the developing cerebellum of the rat is associated with strong c-Jun expression and formation of high molecular weight c-Jun complexes. J Neuropathol Exp Neurol. 1997;56:1–9.

90. Daval JL, Pourie G, Grojean S, Lievre V, Strazielle C, Blaise S, et al. Neonatal hypoxia triggers transient apoptosis followed by 224 EPMA Journal (2011) 2:211–230 neurogenesis in the rat CA1 hippocampus. Pediatr Res.2004;55:561–7.

91. Blomgren K, Zhu C, Wang X, Karlsson JO, Leverin AL, Bahr BA, et al. Synergistic activation of caspase-3 by m-calpain after neonatal hypoxia-ischemia: a mechanism of "pathological apoptosis"? J Biol Chem. 2001;276:10191–8.

92. Fatemi A, Wilson MA, Johnston MV. Hypoxic-ischemic encephalopathy in the term infant. Clin Perinatol. 2009;36:835–58. vii.

93. Johnston MV, Fatemi A, Wilson MA, Northington F. Treatment advances in neonatal neuroprotection and neurointensive care. Lancet Neurol. 2011;10:372–82

94. Novelli A, Reilly JA, Lysko PG, Henneberry RC. Glutamate becomes neurotoxic via the N methyl-D-aspartate receptor when intracellular energy levels are reduced. Brain Res. 1988;451:205–12.

95. Choi DW. Glutamate neurotoxicity and diseases of the nervous system. Neuron. 1988;1:62334.

96. Chen Y, Herrera-Marschitz M, Bjelke B, Blum M, Gross J, Andersson K. Perinatal asphyxia induced changes in rat brain tyrosine hydroxylase-immunoreactive cell body number: effects of nicotine treatment. Neurosci Lett. 1997;221:77–80.

97. Johnston MV. Cellular alterations associated with perinatal asphyxia. Clin Invest Med. 1993;16:122–32.

98. Yeh TH, Hwang HM, Chen JJ, Wu T, Li AH, Wang HL. Glutamate transporter function of rat hippocampal astrocytes is impaired following the global ischemia. Neurobiol Dis. 2005;18:476–83.

99. Silverstein F, Johnston MV. Effects of hypoxia-ischemia on monoamine metabolism in the immature brain. Ann Neurol. 1984;15:342–7.

100. SiesjöBK,Katsura K, Pahlmark K, SmithM-L. The multiples causes of ischemic brain damage: a speculative synthesis. In: Krieglstein J, Oberpichler-Schwenk H, editors. Pharmacology of cerebral ischemia. Stuttgart: Medpharm Scientific Publishers; 1992. p. 511–525

101. Olney JW. Brain lesions, obesity, and other disturbances in mice treated with monosodium glutamate. Science.1969;164:719–21.

102. Benveniste H, Drejer J, Schousboe A, Diemer NH. Elevation of the extracellular concentrations of glutamate and aspartate in rat hippocampus during transient cerebral ischemia monitored by intracerebral microdialysis. J Neurochem. 1984;43:1369–74.

103. McDonald JW, Johnston MV. Pharmacology of N-methyl-Daspartate- induced brain injury in an in vivo perinatal rat model. Synapse. 1990;6:179–88.

104. Pulsinelli WA, Brierley JB, Plum F. Temporal profile of neuronal damage in a model of transient forebrain ischemia. Ann Neurol. 1982;11:491–8.

105. Rothman SM, Olney JW. Glutamate and the pathophysiology of hypoxic–ischemic brain damage. Ann Neurol .1986;19:105–11.

106. Chen HL, Pistollato F, Hoeppner DJ, Ni HT, McKay RD, Panchision DM. Oxygen tension regulates survival and fate of mouse central nervous system precursors at multiple levels. Stem Cells. 2007;25:2291–301.

107. Riikonen RS, Kero PO, Simell OG. Excitatory amino acids in cerebrospinal fluid in neonatal asphyxia. Pediatr Neurol. 1992;8:37–40.

108. Hagberg H, Thornberg E, Blennow M, Kjellmer I, Lagercrantz H, Thiringer K, et al. Excitatory amino acids in the cerebrospinal fluid of asphyxiated infants: relationship to hypoxic-ischemic encephalopathy. Acta Paediatr. 1993;82:925–9.

109. Monyer H, Burnashev N, Laurie DJ, Sakmann B, Seeburg PH. Developmental and regional expression in the rat brain and functional properties of four NMDA receptors. Neuron. 1994;12:529–40.

110. Guerguerian AM, Brambrink AM, Traystman RJ, Huganir RL, Martin LJ. Altered expression and phosphorylation of N-methyl-D-aspartate receptors in piglet striatum after hypoxia ischemia. Brain Res Mol Brain Res. 2002;104:66–80.

111. Mueller-Burke D, Koehler RC, Martin LJ. Rapid NMDA receptor phosphorylation and oxidative stress precede striatal neurodegeneration after hypoxic ischemia in newborn piglets and are attenuated with hypothermia. Int J Dev Neurosci. 2008;26:67–76.

112. Sanchez RM, Koh S, Rio C, Wang C, Lamperti ED, Sharma D, et al. Decreased glutamate receptor 2 expression and enhanced epileptogenesis in immature rat hippocampus after perinatal hypoxia-induced seizures. J Neurosci. 2001;21:8154–63.

113. Talos DM, Fishman RE, Park H, Folkerth RD, Follett PL, Volpe JJ, et al. Developmental regulation of alpha-amino-3-hydroxy-5- methyl-4-isoxazole-propionic acid receptor subunit expression in forebrain and relationship to regional susceptibility to hypoxic/ ischemic injury. I. Rodent cerebral white matter and cortex. J Comp Neurol. 2006;497:42–60.

114. Beattie MS, Ferguson AR, Bresnahan JC. AMPA-receptor trafficking and injury-induced cell death. Eur J Neurosci. 2010;32:290–7.

115. Silverstein FS, Torke L, Barks J, Johnston MV. Hypoxiaischemia produces focal disruption of glutamate receptors in developing brain. Brain Res. 1987;431:33–9.

116. McDonald JW, Silverstein FS, Johnston MV. MK-801 protects the neonatal brain from hypoxic-ischemic damage. Eur J Pharmacol. 1987;140:359–61.

117. Ford LM, Sanberg PR, Norman AB, Fogelson MH. MK-801 prevents hippocampal neurodegeneration in neonatal hypoxicischemic rats. Arch Neurol. 1989;46:1090–6.

118. Volbracht C, van Beek J, Zhu C, Blomgren K, Leist M. Neuroprotective properties of memantine in different in vitro and in vivo models of excitotoxicity. Eur J Neurosci. 2006;23:2611–22.

119. Bergles DE, Jahr CE. Synaptic activation of glutamate transporters in hippocampal astrocytes. Neuron. 1997;19:1297–308.

120. Carmignoto G. Reciprocal communication systems between astrocytes and neurones. Prog Neurobiol. 2000;62:561–81.

121. Domingues AM, Taylor M, Fern R. Glia as transmitter sources and sensors in health and disease. Neurochem Int. 2010;57:359–66.

122. Hamilton NB, Attwell D. Do astrocytes really exocytose neurotransmitters? Nat Rev Neurosci. 2010;11:227–38.

123. Halassa MM, Haydon PG. Integrated brain circuits: astrocytic networks modulate neuronal activity and behavior. Annu Rev Physiol. 2010;72:335–55.

124. Frizzo JK, Cardoso MP, de Assis AM, Perry ML, Volonte C, Frizzo ME. Effects of acute perinatal asphyxia in the rat hippocampus. Cell Mol Neurobiol. 2010;30:683–92.

125. Jantzie LL, Cheung PY, Johnson ST, Bigam DL, Todd KG. Cerebral amino acid profiles after hypoxia-reoxygenation and Nacetylcysteine treatment in the newborn piglet. Neonatology. 2010;97:195–203.

126. Dallas M, Boycott HE, Atkinson L, Miller A, Boyle JP, Pearson HA, et al. Hypoxia suppresses glutamate transport in astrocytes. J Neurosci. 2007;27:3946–55.

127. Holopainen IE, Lauren HB. Glutamate signaling in the pathophysiology and therapy of prenatal insults. PharmacolBiochemBehav. 2011; doi:10.1016/j.pbb.2011.03.016.

128. Van den Heuvel DM, Pasterkamp RJ. Getting connected in the dopamine system. Prog Neurobiol. 2008;85:75–93.

129. Pastuszko A. Metabolic responses of the dopaminergic system during hypoxia in newborn brain. Biochem Med Metabol Biol. 1994;51:1–15.

130. Broderick PA, Gibson GE. Dopamine and serotonin in rat striatum during in vivo hypoxic hypoxia. Metab Brain Dis. 1989;4:143–53.

131. Akiyama Y, Ito A, Koshimura K, Ohue T, Yamagata S, Miwa S, et al. Effects of transient forebrain ischemia and reperfusion on function of dopaminergic neurons and dopamine reuptake in vivo in rat striatum. Brain Res. 1991;561:120–7.

132. Akiyama Y, Koshimura K, Ohue T, Lee K, Miwa S, Yamagata S, et al. Effects of hypoxia on the activity of the dopaminergic neuron system in the rat striatum as studied by in vivo brain microdialysis. J Neurochem. 1991;57:997–1002.

133. Knapp AG, Dowling JE. Dopamine enhances excitatory amino acid-gated conductances in cultured retinal horizontal cells. Nature. 1987;325:437–9.

134. Globus MY, Busto R, Dietrich WD, Martinez E, Valdes I, Ginsberg MD. Effect of ischemia on the in vivo release of striatal dopamine, glutamate, and gamma-aminobutyric acid studied by intracerebral microdialysis. J Neurochem. 1988;51:1455–64.

135. Chen Y, Engidawork E, Loidl F, Dell'Anna E, Goiny M, Lubec G, et al. Short- and long-term effects of perinatal asphyxia on monoamine, amino acid and glycolysis product levels measured in the basal ganglia of the rat. Brain Res Dev Brain Res. 1997;104:19–30.

136. Saugstad OD. Hypoxanthine as an indicator of hypoxia: its role in health and disease through free radical production. Pediatr Res. 1988;23:143–50.

137. Goplerud JM, Mishra OP, Delivoria-Papadopoulos M. Brain cell membrane dysfunction following acute asphyxia in newborn piglets. Biol Neonate. 1992;61:33–41.

138. Floyd RA. Role of oxygen free radicals in carcinogenesis and brain ischemia. FASEB J. 1990;4:2587–97.

139. Olano M, Song D, Murphy S, Wilson DF, Pastuszko A. Relationships of dopamine, cortical oxygen pressure, and hydroxyl radicals in brain of newborn piglets during hypoxia and posthypoxic recovery. J Neurochem. 1995;65:1205–12.

140. Halliwell B, Gutteridge JM. The importance of free radicals and catalytic metal ions in human diseases. Mol Aspects Med. 1985;8:89–193.

141. Gross J, Andersson K, Chen Y, Muller I, Andreeva N, Herrera- Marschitz M. Effect of perinatal asphyxia on tyrosine hydroxylase and D2 and D1 dopamine receptor mRNA levels expressed during early postnatal development in rat brain. Brain Res Mol Brain Res. 2005;134:275–81.

142. Morales P, Klawitter V, Johansson S, Huaiquin P, Barros VG, Avalos AM, et al. Perinatal asphyxia impairs connectivity and dopamine neurite branching in organotypic triple culture from rat substantia nigra, neostriatum and neocortex. Neurosci Lett. 2003;348:175–9.

143. Klawitter V, Morales P, Bustamante D, Gomez-Urquijo S, Hokfelt T, Herrera-Marschitz M. Plasticity of basal ganglia neurocircuitries following perinatal asphyxia: effect of nicotinamide. Exp Brain Res. 2007;180:139–52.

144. Strackx E, Van den Hove DL, Steinbusch HP, Steinbusch HW, Vles JS, Blanco CE, et al. A combined behavioral and morphological study on the effects of fetal asphyxia on the nigrostriatal dopaminergic system in adult rats. Exp Neurol. 2008;211:413–22.

145. Derijck AA, Van Erp S, Pasterkamp RJ. Semaphorin signaling: molecular switches at the midline. Trends Cell Biol. 2010;20:568–76.

146. Seiger A, Olson L. Late prenatal ontogeny of central monoamine neurons in the rat: Fluorescence histochemical observations. Z AnatEntwicklungsgesch. 1973;140:281–318.

147. Antonopoulos J, Dori I, Dinopoulos A, Chiotelli M, Parnavelas JG. Postnatal development of the dopaminergic system of the striatum in the rat. Neuroscience. 2002;110:245–56.

148. Kohlhauser C, Kaehler S, Mosgoeller W, Singewald N, Kouvelas D, Prast H, et al. Histological changes and neurotransmitter levels three months following perinatal asphyxia in the rat. Life Sci. 1999;64:2109–24.

149. Pasterkamp RJ, Kolodkin AL. Semaphorin junction: making tracks toward neural connectivity. CurrOpinNeurobiol. 2003;13:79–89.

150. Klawitter V, Morales P, Bustamante D, Goiny M, Herrera- Marschitz M. Plasticity of the central nervous system (CNS) following perinatal asphyxia: does nicotinamide provide neuroprotection? Amino Acids. 2006;31:377–84.

151. Herrera-Marschitz M, Kohlhauser C, Gomez-Urquijo S, Ubink R, Goiny M, Hokfelt T. Excitatory amino acids, monoamine, and nitric oxide synthase systems in organotypic cultures: biochemical and immunohistochemical analysis. Amino Acids. 2000;19:33–43.

152. Gomez-Urquijo SM, Hokfelt T, Ubink R, Lubec G, Herrera- Marschitz M. Neurocircuitries of the basal ganglia studied in organotypic cultures: focus on tyrosine hydroxylase, nitric oxide synthase and neuropeptide immunocytochemistry. Neuroscience. 1999;94:1133–51.

153. Morales P, Simola N, Bustamante D, Lisboa F, Fiedler J, Gebicke-Haerter PJ, et al. Nicotinamide prevents the longterm effects of perinatal asphyxia on apoptosis, non-spatial working memory and anxiety in rats. Exp Brain Res. 2010;202:1–14.

154. Sanders MJ, Wiltgen BJ, Fanselow MS. The place of the hippocampus in fear conditioning. Eur J Pharmacol. 2003;463:217–23.

155. Kalisch R, Schubert M, Jacob W, Kessler MS, Hemauer R, Wigger A, et al. Anxiety and hippocampus volume in the rat. Neuropsychopharmacology. 2006;31:925–32.

156. Kohlhauser C, Kaehler S, Mosgoeller W, Singewald N, Kouvelas D, Prast H, et al. Histological changes and neurotransmitter levels three months following perinatal asphyxia in the rat. Life Sci. 1999;64:2109–24.3

157. Klawitter V, Morales P, Johansson S, Bustamante D, Goiny M, Gross J, et al. Effects of perinatal asphyxia on cell survival, neuronal phenotype and neurite growth evaluated with organotypic triple cultures. Amino Acids. 2005;28:149–55.

158. Ziebell JM, Morganti-KossmannMC. Involvement of pro- and anti-inflammatory cytokines and chemokines in the pathophysiology of traumatic brain injury. Neurotherapeutics. 2010;7:22–30.

159. Lehnardt S, Lehmann S, Kaul D, Tschimmel K, Hoffmann O, Cho S, et al. Toll-like receptor 2 mediates CNS injury in focal cerebral ischemia. J Neuroimmunol. 2007;190:28–33.

160. Glass CK, Saijo K, Winner B, Marchetto MC, Gage FH. Mechanisms underlying inflammation in neurodegeneration. Cell. 2010;140:918–34.

161. Tracey KJ. Physiology and immunology of the cholinergic antiinflammatory pathway. J Clin Invest. 2007;117:289–96.

162. Greaves DR, Gordon S. Macrophage-specific gene expression: current paradigms and future challenges. Int J Hematol. 2002;76:6–15.

163. Monif M, Burnstock G, Williams DA. Microglia: proliferation and activation driven by the P2X7 receptor. Int J Biochem Cell Biol. 2010;42:1753–6.

164. Harry GJ, Kraft AD. Neuroinflammation and microglia: considerations and approaches for neurotoxicity assessment. Expert Opin Drug MetabToxicol. 2008;4:1265–77.

165. Giulian D, Vaca K. Inflammatory glia mediate delayed neuronal damage after ischemia in the central nervous system. Stroke. 1993;24:I84–90.

166. Hermoso MA, Cidlowski JA. Putting the brake on inflammatory responses: the role of glucocorticoids. IUBMB Life. 2003;55:497–504.

167. Clemens JA, Stephenson DT, Yin T, Smalstig EB, Panetta JA, Little SP. Drug-induced neuroprotection from global ischemia is associated with prevention of persistent but not transient activation of nuclear factor-kappaB in rats. Stroke. 1998;29:677–82.

168. Herrmann O, Baumann B, de Lorenzi R, Muhammad S, Zhang W, Kleesiek J, et al. IKK mediates ischemia-induced neuronal death. Nat Med. 2005;11:1322–9.

169. Lubec B, Labudova O, Hoeger H, Kirchner L, Lubec G. Expression of transcription factors in the brain of rats with perinatal asphyxia. Biol Neonate. 2002;81:266–78.

170. Buller KM, Carty ML, Reinebrant HE, Wixey JA. Minocycline: a neuroprotective agent for hypoxic-ischemic brain injury in the neonate? J Neurosci Res. 2009;87:599–608.

171. Battista D, Ferrari CC, Gage FH, Pitossi FJ. Neurogenic niche modulation by activated microglia: transforming growth factor beta increases neurogenesis in the adult dentate gyrus. Eur J Neurosci. 2006;23:83–93.

172. Hanisch UK, Kettenmann H. Microglia: active sensor and versatile effector cells in the normal and pathologic brain. Nat Neurosci. 2007;10:1387–94.

173. Girard S, Kadhim H, Roy M, Lavoie K, Brochu ME, Larouche A, et al. Role of perinatal inflammation in cerebral palsy. Pediatr Neurol. 2009;40:168–74.

174. Foster-Barber A, Dickens B, Ferriero DM. Human perinatal asphyxia: correlation of neonatal cytokines with MRI and outcome. Dev Neurosci. 2001;23:213–8.

175. Aly H, Khashaba MT, El-Ayouty M, El-Sayed O, Hasanein BM. IL-1beta, IL-6 and TNF-alpha and outcomes of neonatal hypoxic ischemic encephalopathy. Brain Dev. 2006;28:178–82.

176. Deng W. Neurobiology of injury to the developing brain. Nat Rev Neurol. 2010;6:328–36.

177. Alano CC, Ying W, Swanson RA. Poly(ADP-ribose) polymerase-1-mediated cell death in astrocytes requires NAD+ depletion and mitochondrial permeability transition. J Biol Chem. 2004;279:18895–902.

178. Leppard JB, Dong Z, Mackey ZB, Tomkinson AE. Physical and functional interaction between DNA ligase IIIalpha and poly (ADP-Ribose) polymerase 1 in DNA single-strand break repair. Mol Cell Biol. 2003;23:5919–27.

179. Mishra OP, Akhter W, Ashraf QM, Delivoria-Papadopoulos M. Hypoxia-induced modification of poly (ADP-ribose) polymerase and dna polymerase beta activity

in cerebral cortical nuclei of newborn piglets: role of nitric oxide. Neuroscience. 2003;119:1023–32.

180. Wilson SH. Mammalian base excision repair and DNA polymerase beta. Mutat Res. 1998;407:203–15.

181. Chiappe-Gutierrez M, Kitzmueller E, Labudova O, Fuerst G, Hoeger H, Hardmeier R, et al. mRNA levels of the hypoxia inducible factor (HIF-1) and DNA repair genes in perinatal asphyxia of the rat. Life Sci. 1998;63:1157–67.

182. Sung P, Bailly V, Weber C, Thompson LH, Prakash L, Prakash S. Human xeroderma pigmentosum group D gene encodes a DNA helicase. Nature. 1993;365:852–5.

183. de Murcia G, Menissier de Murcia J. Poly(ADP-ribose) polymerase: a molecular nick-sensor. Trends Biochem Sci. 1994;19:172–6.

184. Hortobagyi T, Gorlach C, Benyo Z, Lacza Z, Hortobagyi S, Wahl M, et al. Inhibition of neuronal nitric oxide synthase-mediated activation of poly(ADP-ribose) polymerase in traumatic brain injury: neuroprotection by 3-aminobenzamide. Neuroscience. 2003;121:983–90.

185. Altmeyer M, Hottiger MO. Poly(ADP-ribose) polymerase 1 at the crossroad of metabolic stress and inflammation in aging. Aging (Albany NY). 2009;1:458–69.

186. Poitras MF, Koh DW, Yu SW, Andrabi SA, Mandir AS, Poirier GG, et al. Spatial and functional relationship between poly(ADPribose) polymerase-1 and poly(ADP-ribose) glycohydrolase in the brain. Neuroscience. 2007;148:198–211.

187. Rouleau M, Patel A, Hendzel MJ, Kaufmann SH, Poirier GG. PARP inhibition: PARP1 and beyond. Nat Rev Cancer. 2010;10:293–301.

188. Haile WB, Echeverry R, Wu F, Guzman J, An J, Wu J, et al. Tumor necrosis factor-like weak inducer of apoptosis and fibroblast growth factor-inducible 14 mediate cerebral ischemiainducedpoly(ADP-ribose) polymerase-1 activation and neuronal death. Neuroscience. 2010;171:1256–64.

189. D'Amours D, Sallmann FR, Dixit VM, Poirier GG. Gain-offunction of poly(ADP-ribose) polymerase-1 upon cleavage by apoptotic proteases: implications for apoptosis. J Cell Sci. 2001;114:3771–8.

190. Seidl R, Stockler-Ipsiroglu S, Rolinski B, Kohlhauser C, Herkner KR, Lubec B, et al. Energy metabolism in graded perinatal asphyxia of the rat. Life Sci. 2000;67:421–35.

191. Burkle A. Physiology and pathophysiology of poly(ADPribosyl) ation. Bioessays. 2001;23:795–806.

192. Lorek A, Takei Y, Cady EB, Wyatt JS, Penrice J, Edwards AD, et al. Delayed ("secondary") cerebral energy failure after acute hypoxia-ischemia in the newborn piglet: continuous 48-hour studies by phosphorus magnetic resonance spectroscopy. Pediatr Res. 1994;36:699–706.

193. Yoles E, Zarchin N, Zurovsky Y, Mayevsky A. Metabolic and ionic responses to global brain ischemia in the newborn dog in vivo: II. Post-natal age aspects. Neurol Res. 2000;22:623–9.

194. Mayevsky A, Rogatsky GG. Mitochondrial function in vivo evaluated by NADH fluorescence: from animal models to human studies. Am J Physiol Cell Physiol. 2007;292:C615–40.

195. Moroni F. Poly(ADP-ribose)polymerase 1 (PARP-1) and postischemic brain damage. CurrOpinPharmacol. 2008;8:96–103.

196. Szabo C. Cardioprotective effects of poly(ADP-ribose) polymerase inhibition. Pharmacol Res. 2005;52:34–43.

197. Grupp IL, Jackson TM, Hake P, Grupp G, Szabo C. Protection against hypoxia-reoxygenation in the absence of poly (ADPribose) synthetase in isolated working hearts. J Mol Cell Cardiol. 1999;31:297–303.

ANNEXURE

ANNEXURE I

ETHICAL CLEARANCE CETIFICATE

Institutional Ethical Committee * BLDE U's Shri B.M. Patil Medical College *
BIJAPUR-586 103.
OUTWARD
No. 64/17
Date 27/2/2017

B.L.D.E. UNIVERSITY'S
SHRI.B.M.PATIL MEDICAL COLLEGE, BIJAPUR-586 103
INSTITUTIONAL ETHICAL COMMITTEE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this college met on 22-02-2017 *at* 3-30pm *to scrutinize the Synopsis of Postgraduate Students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected & revised version synopsis of the Thesis has been accorded Ethical Clearance.*

Title "Clinical and Hematological Markers to predict Short term outcome in birth asphyxia"

Name of P.G. student Dr Sharath Keerthy R.
Dept of Pediatrics

Name of Guide/Co-investigator Dr S.V. Patil. Prof & HOD.
Dept of Pediatrics

Tejaswini

DR.TEJASWINI. VALLABHA
CHAIRMAN
INSTITUTIONAL ETHICAL COMMITTEE
BLDEU'S, SHRI.B.M.PATIL
MEDICAL COLLEGE, BIJAPUR.

Following documents were placed before E.C. for Scrutinization
1) Copy of Synopsis/Research project.
2) Copy of informed consent form
3) Any other relevant documents.

ANNEXURE II

RESEARCH INFORMED CONSENT FORM

"CLINICAL AND HEMATOLOGICAL MARKERS TO PREDICT SHORT TERM OUTCOME IN BIRTH ASPHYXIA."

PURPOSE OF RESEARCH:

The present study will help in assessing risk factors of birth asphyxia and its short-term outcome can help in predictive diagnosis and personalized therapeutic interventions.

PROCEDURE:

I understand that after having obtained a sample of Umbilical cord blood as well as venous blood and a detailed clinical history, thorough clinical examination and relevant investigations, a final follow up of the birth asphyxia neonate and its outcome is planned.

RISK AND DISCOMFORTS:

None

BENEFITS:

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

CONFIDENTIALITY:

I understand that the medical information produced by this study will become a part of hospital records and will be subject to the 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 the permission.

REQUEST FOR MORE INFORMATION:

I understand that I may ask more questions about the study at any time;

`Dr. Sharath Keerthy R 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. Sharath Keerthy R may terminate my participation in the study after he has explained the reasons for doing so.

INJURY STATEMENT:

I understand that in the unlikely event of injury to my baby resulting directly from baby's participation in this study, if such injury were reported promptly, the appropriate treatment would be available to the baby. 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. Sharath Keerthy R Date

(Investigator)

PARENTS / GUARDIAN CONSENT STATEMENT:

I/We confirm that Dr. Sharath Keerthy R, is doing a study on **"CLINICAL AND HEMATOLOGICAL MARKERS TO PREDICT SHORT TERM OUTCOME IN BIRTH ASPHYXIA."** Dr. Sharath, has explained to us the purpose of research and the study procedure. I/We are willing to give as much as information required for the study and consent for investigations and the possible discomforts as well as benefits. I/We have been explained all the above in detail in our own language and we understand the same. Therefore, we agree to give consent for baby's participate as a subject in this research project.

_____ _____

(Parents / Guardian) Date

_____ _____

(Witness to signature) Date

<u>ಪಾಲಕರು / ಪೋಷಕರು ಒಪ್ಪಿಗೆಯನ್ನು ಸೂಚಿಸುವ ಹೇಳಿಕೆ:</u>

ನಾನು/ನಾವು ಈ ಮೂಲಕ ಧೃಡ ಪಡಿಸುವುದೇನೆಂದರೆ, **ಡಾ. ಶರತ್ ಕೀರ್ತಿ.ಆರ್** ಅವರು **"CLINICAL AND HEMATOLOGICAL MARKERS TO PREDICT SHORT TERM OUTCOME IN BIRTH ASPHYXIA."** ವಿಷಯದ ಮೇಲೆ ಅಧ್ಯಯನ ಮಾಡುತಿದ್ದಾರೆ.

ಡಾ. ಶರತ್ ರವರು ನಮಗೆ ಈ ವಿಷಯದ ಕುರಿತು ಸಂಶೋಧನೆ ನಡೆಸುವ ಉದ್ದೇಶ ಹಾಗು ಅಧ್ಯಯನದ ಕಾರ್ಯ ಪದ್ಧತಿ ಬಗ್ಗೆ ವಿವರಿಸಿರುತ್ತಾರೆ. ನಾನು/ನಾವು ಈ ಅಧ್ಯಯನದ ಸಲುವಾಗಿ ಅವಶ್ಯಕವಿರುವ ಮಾಹಿತಿಗಳನ್ನು ಎಷ್ಟು ಸಾಧ್ಯವೋ ಅಷ್ಟೂ ಮಾಹಿತಿ ನೀಡಲು ಹಾಗು ಈ ಅಧ್ಯಯನದಿಂದ ಆಗಬಹುದಾದ ತೊಂದರೆಗಳು ಹಾಗು ಲಾಭಗಳ ಕುರಿತು ಪತ್ತೆ ಹಚ್ಚಲು ಅವಶ್ಯಕ ಮಾಹಿತಿಗಳನ್ನು ನೀಡಲು ನಮ್ಮ ಸಮ್ಮತಿ ಇರುತ್ತದೆ. ನನಗೆ/ನಮಗೆ ಮೇಲೆ ತಿಳಿಸಿದ ಅಧ್ಯಯನದ ಕುರಿತು ಎಲ್ಲ ಮಾಹಿತಿಗಳನ್ನು ನಮ್ಮ ಮಾತೃಭಾಷೆಯಲ್ಲಿ ವಿವರಿಸಿದ್ದು, ನಾವು ಇದನ್ನು ಅರ್ಥಮಾಡಿಕೊಂಡಿದ್ದೇವೆ. ಆದ್ದರಿಂದ ನಾವು ಈ ಸಂಶೋಧನೆಯಲ್ಲಿನ ಒಂದು ವಿಷಯವಾಗಿ ನಮ್ಮ ಮಗುವಿನ ಭಾಗವಹಿಸುವಿಕೆಯ ಕುರಿತು ಸಮ್ಮತಿಯನ್ನು ನೀಡಿರುತ್ತೇವೆ.

_____ _____

(ತಂದೆತಾಯಿಗಳು / ಪೋಷಕರು) ದಿನಾಂಕ

_____ _____

ಸಾಕ್ಷಿದಾರರ ಸಹಿ ದಿನಾಂಕ

ANNEXURE- III

PROFORMA

Name: B/o IP No:

Father's Name(*with address and Phone Number*):

DOB/Sex:Out born/Inborn

Complaints at Presentation:Apgar score:

Baby details:

1. Gestational age:
2. Chronological Age at presentation:
3. Birth weight:
4. Anthropometry:

   HC:CC:

   Length: US:LS-

Diagnosis:

Maternal Details:

1. Age: years
2. Weight: Kg(present)
3. Height: cm
4. Educational status: Illiterate/ Primary/ Secondary/ High School/ Degree/ PG
5. Occupation: Housewife/ Unskilled Labor/ Skilled worker

   Professional(Specify)
6. Married life/ Consanguinity: Yes/No. If Yes Degree:
7. Pregnancy Weight Gain:
8. Pre-Pregnancy Wt(*if available*):

Obstetric History:

1. Parity
2. H/o Still Births/Miscarriages/Death of Baby: Yes/No.If yes specify,
3. H/o previously Asphyxiated Baby: Yes/No.
4. Infertility Treatment: Yes/No. If yes specify therapy:

Antenatal History:

1. ANC Check up:
    a) Booking Status: Yes/No, If Yes, hospital-
    b) No of Antenatal Visits:
    c) No of Antenatal USG and when done:

Report*(if available)*:

    d) Iron & Folic Acid Tab Supplements': Yes/No. If yes duration,
    e) Inj TT Taken: Yes/No:

2. Medical Conditions during Present Pregnancy:
    a) Hypertension: Yes/No, If Yes, max BP- Symp/ Asymptomatic
    b) Edema: Yes/No,If yes Specify with Duration and extension
    c) Diabetes Mellitus/ Gestational DM:Yes/No. If yes specify--Highest blood Glucose level:
        -Insulin Units
        -Duration of Treatment
    d) Anemia: Yes/No If Yes,
        -Lowest Hb %
        -Drugs Taken
        -Any Blood Transfusion. Yes/ No; If yes specify no of times-

e) Thyroid Disorders: Yes/No If Yes,

-Diagnosed at age:

-Drugs Taken

f)Seizure Disorder: Yes/No If Yes,

-Diagnosed at age:

-No of times

-Drugs Taken with duration:

g) Cardiac Disorder: Yes/No. If yes specify-

h) Others(*Infection*): Yes/No. If yes specify- Diagnosis

-Term

-Duration

Intra partum Factors:

1. Place of delivery:
2. Type of delivery:

-Normal/ Instrumental:If instrumental, specify

-C-Section: If Yes; -Emergency/ Elective

-Indication:

-Anesthesia:

3. Oxytocin Augmented Labor: Yes/ No
4. Premature Rupture Of Membranes: Yes/No.

- If yes duration before delivery-

5. Malpresentations: Yes/No. If yes specify-
6. Cord Accidents :Yes/No. If yes specify-

-Cord Presentations

-Cord Prolapse

-Umbilical cord Presentation

-Cord Around Neck

-True Knot/ Pseudo Knot

7. ChorioAmnionitis: Yes/No.
8. Meconium stained Liqour :Yes/No.
9. Reduced Fetal Movements: Yes/No. If yes duration before delivery-

10. Complications during Delivery: Yes/No. If yes specify-
    -Bleeding from vagina
    -Smelly Excessive Vaginal Discharge
    -Edema Of Body Parts: Yes/No. If yes specify part
    -Severe Or Persistent Abdomen or Body pain
    -Arrest Of Labor: Yes/No. If yes specify stage & duration:

11. Failed Instrumental Delivery: Yes/No. If yes specify

Fetal risk factors:

1. LBW: Yes/No. If yes specify wt-
2. IUGR:Yes/No. If yes Symmetrical or Asymmetrical-
3. Birth Injury: Yes/No. If yes specify-
4. Apgar Score(*if known*): At 1min:At 5min:At 10min
5. Resusitative Measures: O_2/ O_2 with Bag & Mask/ O2 with Bag & Tube/ Cardiac Percussion/Drugs (specify)
6. Duration of shifting: <12 hours / >12 hours (Specify)

Placental risk factors:

1. Placental Infarct: Yes/No. If yes specify-
2. Placenta Previa :Yes/No.
3. Abruptio Placenta: Yes/No.
4. Others: specify-

Haematological Factors:

1. Sample : Cord Blood/Peripheral Blood
2. Nucleated RBC Count/ 100 WBC :
3. Absolute Nucleated RBC count/mm^3 :
4. Reticulocyte count:
5. CBC:6.Others:

Medical conditions of mother: Yes/No. If yes specify-

Socio economic status of Parents:

1. Father education: Illiterate/ Primary/ Secondary/ High School/ Degree/ PG
2. Father occupation: Unskilled Labor/ Skilled worker/Professional(Specify)
3. Source of Drinking water: specify
4. Toilet Facilities: Yes/ No
5. Socio-economic status(*as per B G Prasad scale*):

Outcome:

1. Death/ Discharge/ Discharge Against Medical Advice(DAMA)

   If Death; Cause-If DAMA; Cause-
2. Duration of NICU Stay:

Problems in NICU:

1. Hypotension: Yes/No.
2. Hypothermia: Yes/No.
3. Hypoglycemia: Yes/No. If yes duration of treatment-
4. Seizures: Yes/No. If yes type and age at first convultion-

   <12hrs(with no of times)>12 hrs(with no of time

   -Drugs used for convulsions with duration:
5. Support needed:

SUPPORT	No Of Days
O2	
CPAP	
VENTILLATOR	

6. Hyperbilirubinemia: Yes/No. If yes day/ duration/ Highest level-

7. Activity-

   Hyperactive: Yes/No; If yes, Duration:

   Lethargy: Yes/No; If yes, Duration:

8. Feeding Pattern:

   Tube feeding: Yes/No; If yes, Duration:

   Spoon feeding: Yes/No; If yes, Duration:

   DBF:Yes/No; If yes, Duration:

9. Coma: Yes/No; If yes, Duration:

10. Any other specify-

Neurological status at discharge:

Clinical (Neonatal Neurological Examination):

TESTS	**NORMAL**	**RED FLAGS**
Mental Status	Alert, Cries when Awake	Irritable or Lethargic
Cranial nerves -Pupils -Hearing -Suck+Swallow	Fix and Follow Eyes turn to sound Coordinated Suck+Swallow	Unequal/don't follow light No response to sound. "Chomp Suck"*(Clamps Down on pacifier and no suck)*
Motor System	Flexed in all extremities	-Hypotonic -Hypertonic -Writhing movements
Reflexes	-Full Moro -Asym tonic neck reflex -Strong Grasp *(Able to lift out of bed)*	-Asymmetry -Obligatory or Sustained *(Pyramidal or Extra pyramidal involvement)* -Fixed Obligate Grasp *(Suggests B Hemispheric Dysfunction)*

Investigations:

i. Neurosonogram:

ii. CT/MRI:

Any Other Info:

1. Person who Conducted Delivery: Doctor/Nurse/ Midwife
2. Person who First Resuscitated the baby: Doctor/Nurse/ Midwife
3. Resuscitation measures done outside: O_2/ O_2 with Bag & Mask/ O2 with Bag & Tube/ Cardiac Percussion/Drugs (specify)

ANNEXURE- IV

KEY TO MASTER CHART

OAL	Oxytocin Augmented Labour
MC	Miss Carriage
ND	Normal Delivery
LSCS	C Section
PROM	Premature Rupture of Membranes
CAN	Cord Around Neck
MSAF	Meconium Stained Amniotic Fluid
ABR	Abortion
GDM	Gestational Diabetes Mellitus
LMC	Lower Middle Class
LC	Lower Class
UMC	Upper Middle Class
UC	Upper Class