CORRELATION OF CYTOKINE PATTERN IN MODERATE TO SEVERE PERINATAL ASPHYXIA WITH NEURODEVELOPMENTAL OUTCOME: A PROSPECTIVE COHORT STUDY

THESIS

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NEONATOLOGY

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DECLARATION

I hereby declare that the thesis titled '**Correlation of cytokine pattern in moderate to severe perinatal asphyxia with neurodevelopmental outcome: A Prospective Cohort Study**' embodies the original work carried out by the undersigned in All IndiaInstitute of Medical Sciences, Jodhpur.

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CERTIFICATE

This is to certify that the thesis 'Correlation of cytokine pattern in moderate to severe perinatal asphyxia with neurodevelopmental outcome: A Prospective Cohort Study' is the bonafide work of Dr. Adil Ahmed Khan, carried out under our guidance and supervision, in the Department of Neonatology, All India Institute of Medical Sciences, Jodhpur.

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Dr. Adil Ahmed Khan

Dedicated to

my patients, parents

and teachers...

ABBREVIATIONS

AAP: American Academy of Pediatrics
ACOG: American College of Obstetrics and Gynaecology
AEEG: Amplitude integrated electro-encephalography
AGA: Appropriate for gestational age
AIIMS: All India Institute of Medical Sciences
ANC: Absolute neutrophil count
BBB: Blood-Brain Barrier
BGT: Basal Ganglia and Thalamus
CBF: Cerebral Blood Flow
CHOP: Children Hospital of Philadelphia
CI: Confidence interval
CK-MB: Creatine kinase – myocardial binding
CNS: Central Nervous System
COVID: Corona virus disease
CSF: Cerebrospinal Fluid
CTG: Cardiotocography
CUS: Cranial ultrasound
DWI: Diffusion weighted imaging
EEG: Electroencephalography
EONS: Early onset neonatal sepsis
EPO: Erythropoietin
GDM: Gestational diabetes mellitus
HIE: Hypoxic Ischemic Encephalopathy
HIF: Hypoxia-Inducible Factor
HINE: Hammersmith infantile neurological examination
HNNE: Hammersmith neonatal neurological examination
IAI: Intra-amniotic infection
ICH: Intracranial hemorrhage
IHCP: Intrahepatic cholestasis of pregnancy
IL: Interleukin
IQR: Interquartile range
IVH: Intraventricular hemorrhage
LGA: Large for gestational age
LMICs: Low-Middle Income Countries
LMP: Last menstrual period
LSCS: Lower segment cesarean section
MAS: Meconium Aspiration Syndrome
MCP-1: Monocyte Chemo-attractant Protein
MMPs: Matrix Metallo-proteinases
MRI: Magnetic resonance imaging
NICHD: National Instiute of Child Health and Human Development
NICU: Neonatal intensive care unit
NNF: National Neonatology Forum
NNPD: National Neonatal Perinatal Database
NRP: Neonatal Resuscitation Program
PA: Perinatal Asphyxia
PIH: Pregnancy induced hypertension
PMNs: Polymorpho Nuclear Leukocytes
ROS: Reactive Oxygen Species
SDH: Subdural hemorrhage
SPSS: Statistical package for social sciences
SGA: Small for gestational age
TH: Therapeutic hypothermia
TLC: Total leucocyte count
TNF: Tumor Necrosis Factor
TGF: Transforming growth factor
TOBY: Total body hypothermia

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1. INTRODUCTION

‘Perinatal hypoxic-ischemic encephalopathy (HIE) is a major cause of neonatal death and long-term disability. Approximately 15% to 25% of affected newborns die in the postnatal period and 25% develop severe and permanent neuropsychological sequelae, including cerebral palsy, seizures, visual impairment, mental retardation, learning impairment and epilepsy (1, 2). In developing countries, perinatal asphyxia still remains a leading cause of mortality and later neurodevelopmental disability especially among term neonates. Perinatal asphyxia produces a number of physiologic and biochemical interactions that are complex and intriguing. There are several methods for predicting outcomes in perinatal asphyxia’. ‘Clinical examination, invasive ventilation during resuscitation, APGAR at 10 minutes, EEG, MRI and biomarkers like cord pH, lactate dehydrogenase and free radicals have all been used to predict abnormal neurological outcome in perinatal asphyxia. However there is no single biomarker available at present that accurately predicts poor neurodevelopmental outcome in perinatal asphyxia’ (3, 4). Pro-inflammatory cytokines like interleukin-6 (IL-6) and interleukin −1 beta (IL-1β) are expressed 24 h after the brain insult, which activate microglia and astrocytes which in turn produces more cytokines and chemokines and amplify the immune response leading to brain damage’ (5, 6). ‘HIE injury induces release of cytokines and chemokines, which amplify inflammatory cascades and recruit neutrophils and monocytes to sites of injury’. Serum inflammatory proteins are readily measurable and may be useful biomarkers of phases of injury. Combinations of cytokines are now beginning to be used as early, discriminating predictors of severe brain injury and multi-organ system failure in children’ (7, 8) and in neonates when there is an evidence of early neonatal insult.

‘Cytokines are important inflammatory mediators, and cerebral ischemic injury can trigger a cascade of cytokine induction that acts to orchestrate an in-situ inflammatory reaction (9) and maintains brain tissue homeostasis (10). In general, the roles of cytokines are pleiotropic, and whether the overall effects are pro- or anti-inflammatory in the context of ischemic insults remains controversial. The most studied cytokines related to the inflammatory responses to stroke are IL-1, IL-6, IL-10, tumor necrosis factor-α (TNF-α), and transforming growth factor-β (TGF-β) (11). Despite advances in perinatal care, the outcome of newborns with HIE is poor and the issue still remains challenging in neonatology. The use of an easily approachable and practical biomarker not only could identify neonates with severe brain damage and subsequent adverse outcome, but could also target the group of infants that

would benefit from a neuro-protective intervention in the advised stipulated time' (12). Nowadays MRI brain is used to see any features of brain insult in perinatal asphyxia but MRI brain in itself has limitations of over and under-diagnosing inflammation and HIE insult. Moreover neonatal MRIs when performed in cases of perinatal asphyxia beyond the critical period of inflammation, leads to pseudo-normalization which gives a false impression of normalcy of brain tissue leading to missing the opportunity of early rehabilitation and timely follow-up eventually leading to more percentage of lost to follow up patients. During the COVID era, the major focus again shifted to basics of inflammation and how these parameters can guide in prognostication. Same way in neonatology there is a strong urge to objectify the level of insult secondary to perinatal asphyxia and how much inflammation/injury it has done to brain tissue, so that early intervention and early rehabilitation can be started. Previous studies signaled that flare up of these cytokines post perinatal HIE is a poor prognostic marker which again reinforce the need to measure them at timely intervals initially to check whether the insult's effect got blunted in time without harming the brain tissue or not. Therefore, this study was aimed to evaluate the cytokines (IL-1 beta, IL-6) pattern in newborns with moderate to severe perinatal asphyxia with evidence of HIE in the perinatal period and here we tried to correlate the cytokines pattern with the neurodevelopmental outcome at 3 to 3½ months of age using HINE which is a validated tool for neuro-developmental assessment of infant as early as 2 months. Additionally with this study we tried to correlate the cytokine pattern with the immediate neurological status at the time of discharge (measured throw HNNE optimality scoring) and also with the MRI findings, if any associated. With this study we tried to throw some light on cytokine levels correlation with in-hospital mortality due to perinatal asphyxia and associated neonatal sepsis, if any present. Further, pattern of cytokines and its correlation with HINE is not done in Indian babies previously. The hypothesis of this study was whether the levels of inflammatory mediators will remain persistently elevated for a longer duration in babies who sustain perinatal asphyxia and higher the value worse is the outcome.

Currently there is a debate going on whether to give therapeutic hypothermia in LMICs (13) as many studies are proving therapeutic hypothermia to have deleterious effects in absence of basic and advanced neonatal support thus making the measurement of these cytokines more important and will lead to addition of one more parameter which will help in deciding upon whether there is a need to imminent hypothermia intervention thus helping in quick transfer of such neonates to well equipped health care facilities.

2. REVIEW OF LITERATURE

Perinatal asphyxia – an important issue to address

'Perinatal asphyxia (PA) is a major cause of neonatal and under-five mortality, particularly in developing countries thus leading to significant morbidity. As per the latest estimates, PA accounts for 9.4% (i.e, 0.72 million) of total under-5 mortality worldwide. Along with prematurity and systemic infections, PA is one of the three most common causes of neonatal death' (14, 15).

'It is also an important cause of still births – of the total 2.7 million stillbirths that occur globally, about 1.2 million occurred during the intra-partum period, largely due to asphyxia (16).The National Neonatal Perinatal Database (NNPD; 2002-2003) reported PA to be the commonest cause of still-births, accounting for 45.1% of all such cases (17). Almost all (98.2%) asphyxia-related deaths occur in first week of life, with 73% occurring within 24 hours of birth' (18).

Incidence of perinatal asphyxia

With the improvements in the health care provided to the pregnant women, the incidence of birth asphyxia has declined to levels less than 0.1% in the developed countries, while the developing countries are still having higher levels reaching to 26 per 1000 live births in Nigeria with 40% fatality rate or even more (19-21). 'As per the NNPD (2002-2003), the incidence of PA – defined as Apgar score of <7 at 1 minute of life was 8.4% of all live births. Oxygen was the most commonly used resuscitative measure used in 9.5% of the cases, bag and mask ventilation in 6.3%, chest compression in 0.8% and use of medication in 0.5%. PA was responsible for 28.8% of all the neonatal deaths. Manifestation of hypoxic ischemic encephalopathy (HIE) were seen in approximately 1.4% of live births (17). Asphyxia is a life-long neuromotor disability in a large number of children'.

Definitions

'There is no single, well accepted definition of perinatal asphyxia. The definition is context specific and can be sensitive (e.g. those given by WHO or NNPD for the purpose of deciding immediate care of newborn) or specific (such as the one given by AAP for the purpose of giving a label or predicting the long term outcome)'.

Table 2.1: Definitions and criteria to label perinatal asphyxia and its severity given by different authorities

	Definition
WHO(22)	Failure to initiate and sustain breathing
NNPD Network (78)	'**Moderate PA**: Slow/gasping breathing or an Apgar score of 4 to 6 at 1 minute of life **Severe PA**: No breathing or an Apgar score of 0-3 at 1 minute of age'
AAP and ACOG (23)	'Presence of all of the following criteria: 1. Profound metabolic or mixed academia (pH <7.0) in the umbilical cord blood 2. Persistence of low Apgar score of <3 for more than 5 minutes 3. Signs of neonatal neurological dysfunction (e.g., seizures, encephalopathy, tone abnormalities) 4. Evidence of multiple organ involvement (such as that of kidney, lungs, liver, heart and intestine)'

Etiology:

Etiology of perinatal asphyxia is multifactorial. It is broadly classified on the basis of maternal and fetal/neonatal causes.

Table 2.2: Causal factor for perinatal asphyxia

Maternal	**Fetal/neonatal**
'Placental insufficiency: **1.** Impaired maternal oxygenation **(anemia, pulmonary or cardiac condition or neurologic disease)** **2.** Decreased flow from mother to placenta **(maternal infection, shock, dehydration, hypotension)** **3.** Decreased blood flow from placenta to fetus **(placental abruption, cord prolapse, cord entanglement, cord knot, cord compression, abnormal attachment of cord to placenta)**	**1. 'Failure of oxygenation:** Fetal cyanotic heart disease Severe respiratory distress **2. Severe anemia:** Severe haemorrhage Haemolytic disease Rh isoimmunisation **3. Shock:** Sepsis Massive blood loss (bleeding vasa previa)

4. Impaired gas exchange across placenta or fetal tissue **(maternal hypertension, vascular or connective tissue disorder, maternal diabetes, drug abuse, post-maturity, placental calcification, infarct or fibrosis)** **5.** Increased fetal oxygen requirement **(fetal anemia, fetal infection, IUGR)'**	Intracranial bleed Adrenal hemorrhage'

Table 2.3: Risk factors for perinatal asphyxia

Antepartum risk factors	Intrapartum risk factors
'Maternal diabetes PIH, chronic hypertension Rh isoimmunisation Previous stillbirth Bleeding in 2nd or 3rd trimester (threatened abortion) Maternal infection Polyhydroamnios or oligohydroamnios Post term gestation Multiple gestation Size-date discrepancy Maternal drug abuse Maternal age <16 years or >35 years Unbooked and unsupervised pregnancies'	'Elective or emergency cesarean section Prolonged labour Precipitous labour Prolonged 2nd stage of labour Premature labour Abnormal presentation Rupture of membranes >24 hours Foul smelling amniotic fluid Non reassuring fetal heart rate patterns Use of general anaesthesia Prolapsed cord'

Pathophysiology of perinatal asphyxia:

'The pathophysiology of HIE evolves over hours to days and is marked by 3 phases of injury: 1) acute or primary, 2) latent, and 3) secondary (figure 1). The acute phase is defined by primary energy failure leading to an excitotoxic-oxidative cascade occurring over minutes' (24).

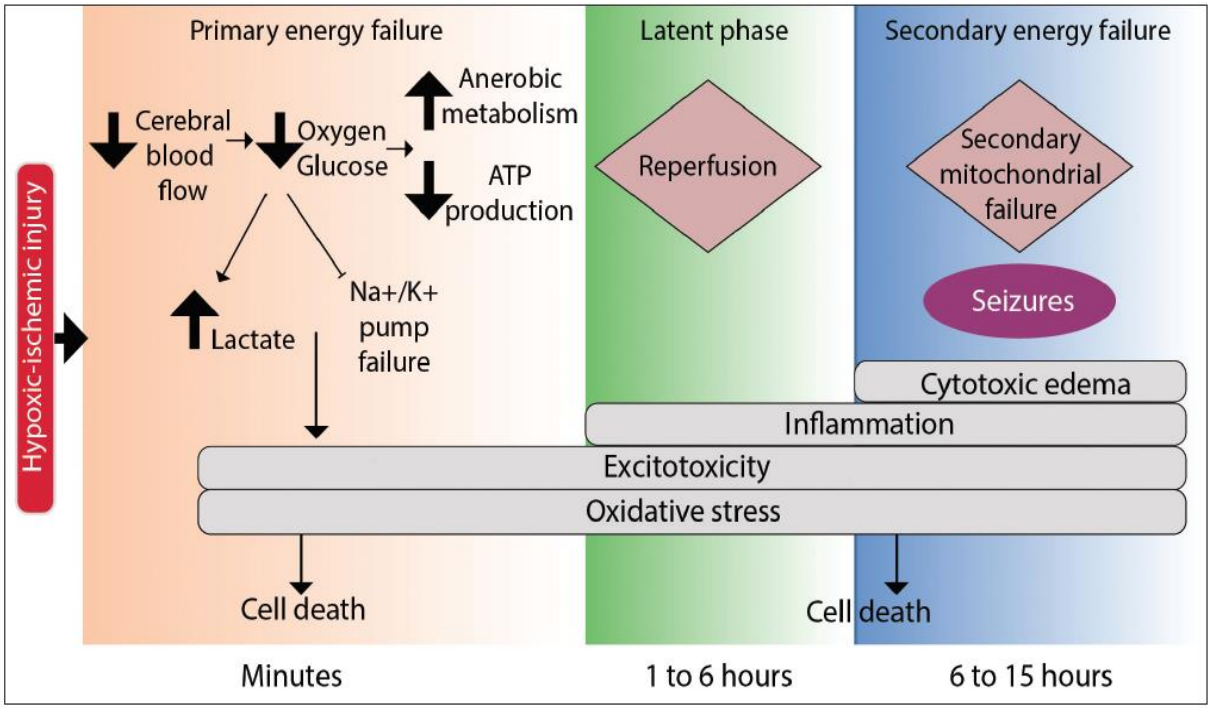


Figure2.1:Phases of HIE insult; Intervening in the latent phase is the most crucial step in the management as it will lead to reduction in inflammation eventually leading to decreased apoptotic cascade which will eventually lead to less residual morbidity and complications**(adopted from article - Therapeutic Hypothermia Effects on Brain Development by Lila T. Worden et al; 2020)**

'Decreased cerebral blood flow and resultant decreased oxygenation leads to cerebral anaerobic metabolism. This metabolic shift induces Na^+/K^+ pump failure causing reduced ATP production, increased lactic acid, and an influx of intracellular calcium and neurotransmitters. Energy failure triggers neuronal depolarization that releases glutamate and reactive oxygen species. These act as excitotoxins disrupting cellular integrity and mitochondrial respiration to cause neuronal cell death'.

'The latent phase is marked by reperfusion and partial recovery of oxidative metabolism 1 to 6 hours after injury, although the length of the latent period varies by the severity of the initial hypoxic-ischemic insult and is shorter in more severe injuries. There is also on-going apoptotic activity from pro-inflammatory signals released during the initial excitotoxic-oxidative cascade from the acute injury'.

'The latent phase is followed by the secondary injury phase, which starts 6 to 15 hours after the initial injury and is marked by delayed energy failure from the recurrent failure of mitochondrial metabolism, cytotoxic edema, and on-going inflammation. [25] The latent phase is considered the optimal window for intervention to mitigate additional neuronal injury and prevent progression to secondary injury'.

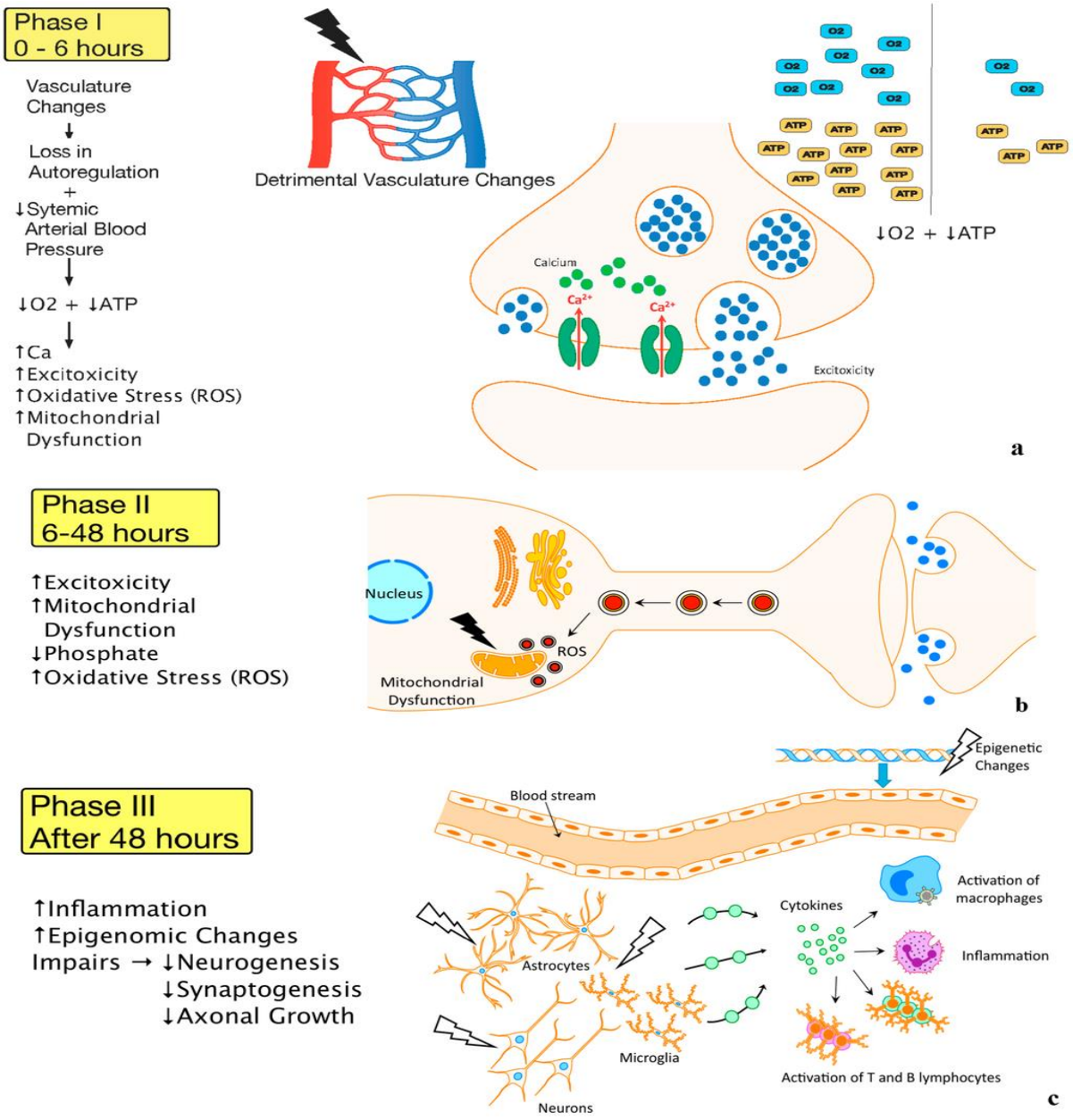
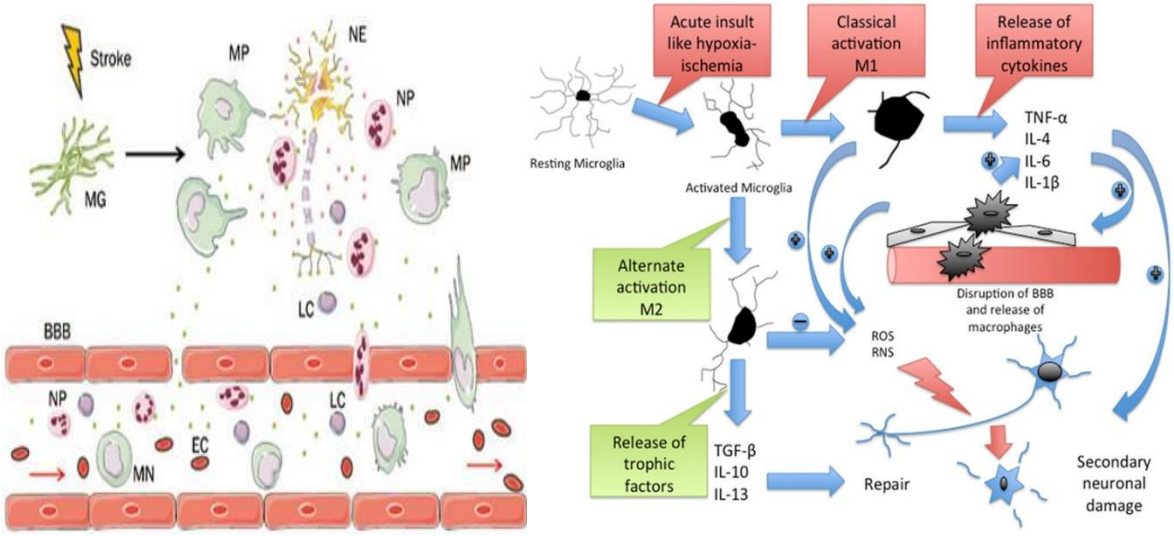


Figure 2.2: 'Various phases of acute and chronic inflammation in HIE (**a**) Vasculature Changes and Primary Energy Failure (Phase I) Legend: A visual representation of the first phase of HIE. Detrimental changes to the vasculature following an HIE insult lead to loss of auto-regulation and severe lowering of the systemic arterial blood pressure. This causes a decrease in oxygen, depletion of ATP, as well as increases in excitotoxicity, intracellular calcium, oxidative stress, and mitochondrial dysfunction; (**b**) Secondary Energy Failure (Phase II). Legend: A schematic representation of the second phase of HIE reveals continued excitotoxicity, oxidative stress, and mitochondrial dysfunction; (**c**) Chronic Inflammation (Phase III). A pictorial representation of the third phase of HIE shows injury to microglia, neurons, and astrocytes leads to continuous release of cytokines and other detrimental factors causing chronic inflammation which in turn leads to epigenetic changes, as well as impairments of synaptogenesis, axonal growth, and neurogenesis.**(adopted from**

Dixon BJ, Reis C, Ho WM, Tang J, Zhang JH. Neuroprotective strategies after neonatal hypoxic ischemic encephalopathy. International journal of molecular sciences. 2015 Sep 15;16(9):22368-401.)' (98)

Role of cytokines in the ischemia-reperfusion injuries of perinatal asphyxia:



A. B.

Figure2.3: 'nflammatory response and role of cytokines in chronic inflammation post HIE insult; A) Schematic diagram of inflammatory responses in HIE. When ischemia occurs, microglia are activated and develop macrophage-like capabilities including phagocytosis, cytokine and chemokine production, antigen presentation and the release of MMPs that weaken the BBB. As a result, peripheral leukocytes infiltrate into the brain, leading to exacerbation of inflammation and neuronal injury. B) Schematic diagram of various cytokines involved in the third phase of HIE insult leading to chronic inflammation. MG, microglia; MP, macrophage; NE, neuron; NP, neutrophil; LC, lymphocyte;MN, monocyte; EC, erythrocyte. **(adopted from Liu F, Mccullough LD. Inflammatory responses in hypoxic ischemic encephalopathy. Acta Pharmacologica Sinica. 2013 Sep;34(9):1121-30'**(99)

Cytokines

'Cytokines are important inflammatory mediators, and cerebral ischemic injury can trigger a cascade of cytokine induction that acts to orchestrate an *in situ* inflammatory reaction (9) and maintains brain tissue homeostasis (10). In general, the roles of cytokines are pleiotropic, and whether the overall effects are pro- or anti-inflammatory in the context of ischemic insults remains controversial even in adult models, for which there are more data than for HIE. The

most studied cytokines related to the inflammatory responses to stroke are IL-1, IL-6, IL-10, tumor necrosis factor-α (TNF-α), and transforming growth factor-β (TGF-β)' (11).

'IL-1β is among the best-characterized early response cytokine and is often expressed concurrently (26). Several types of CNS cells secrete IL-1β, including microglia, astrocytes, and neurons, and this cytokine has potent pro-inflammatory actions. Human newborns with HIE have higher levels of IL-1β in peripheral blood samples compared to normal term neonates, and the IL-1β levels correlate positively with HIE severity (27). The neurotoxic consequences of IL-1 activation have been shown in experimental studies with HIE and other inflammatory disease models (28-33). The most convincing evidence that IL-1 is functionally detrimental in the pathogenesis of HIE is provided by the neuroprotective potential of IL-1 receptor antagonist administration in HIE models in rodents' (34, 35).

Interleukin 1beta (IL-1β):

'IL-1 is subdivided in IL-1α and IL-1β. IL-1β is potent pro-inflammatory cytokine, induced mainly by lymphocytes, macrophages, and monocytes in response to microbial molecules. In addition to the stimulatory effect of the IL-1 family, there are also members IL-1Ra (IL-1 receptor antagonist) that can inhibit or suppress the IL-1 cytokine expression. IL-1Ra is secreted from neutrophils, macrophages, monocytes, dendritic cells and hepatocytes aiming to decrease the inflammation. However, the expression of IL-Ra needs to be expressed up to 1,000-fold in order to efficiently inhibit or suppress the expression of IL-1β (36).During pregnancy IL-1β is also produced by placenta and decidual tissue. The IL-1b and IL-1ra (interleukin1 –receptor antagonist) remain unaltered between 24 and 35 weeks' gestation. At late pregnancy, IL-1a and b concentrations peak at 4 to 14 days prior to labor onset, while IL-1ra decreases with approaching spontaneous term labor. The levels of IL-1beta also increases in preterm labour, pre-eclampsia or other disorder leading to relative ischemia and in intrauterine infection and the balance between the IL-1 and its receptor antagonist help in expecting onset of labor (37). 'IL-1β is an important mediator of pro-inflammatory responses and has been reported to have neurotoxic properties leading to BBB breakdown and apoptotic neuronal death' (38, 39). 'The intracellular half-life ofIL-1β is 2.5 hours and it is released continuously beginning 2 hours after synthesis from monocytes' (40). 'Aly et al. found that the CSF level of IL-1β in term neonates had the highest predictive value of poor neurologic outcome in asphyxia after 6 and 12 months and suggested the central role of IL-1β in the ongoing neuronal injury that occurs in the latent phase following the original hypoxic insult'

(41).'They also found a high CSF to plasma ratio of IL-1β, indicating elevated local production of the cytokine in the CNS. Several animal models also suggest that IL-1β contributes to the brain injury (42, 43). Other studies demonstrated that the deficiency of IL-1β converting enzyme or treatment with IL-1 receptor antagonists (IL-1ra) resulted in the moderation of hypoxic brain injury, decreased post-ischemic edema, and improved neurological outcome. The primary sources of IL-1β are APCs and monocytes, although microglia and endothelial cells are also capable of producing IL-1β. Significant IL-1β production in T lymphocytes is observed in neonates with perinatal asphyxia, which was more pronounced in a severe insult suggesting that CD4+ IL-1β+ cells might play an important role in initiating tissue damage in the brain following the hypoxic insult' (44).

Interleukin-6 (IL-6):

'IL-6 is a pleiotropic cytokine that not only affects the immune system, but also acts in other biological systems and many physiological events, such as regulating cell growth, as well as gene activation, proliferation, survival, and differentiation. IL-6 is produced by a variety of cell types including monocytes, fibroblast, and endothelial cells. Upon stimulation, IL-6 is secreted by many additional cell types including macrophages, T cells, B cells, mast cells, glial cells, eosinophils, keratinocytes, and granulocytes and adipocytes (36). During pregnancy IL-6 is produced by human fetal membranes (amnion and chorion), and its expression increases in both amnion and chorion cells near term, in response to infectious stimuli and in pregnancies with pPROM (45).Literature indicates that the half-life of IL-6 appears to be approximately 1 hour compared with 2 to 3 hours for sIL-6Rsuggesting that sIL-6R, with its slightly longer half-life, may better capture the cumulative IL6 effect because of its receptor activation' (46, 47).

'Several previous studies have associated elevated IL-6 CSF levels with poor neurological outcome, cerebral palsy, and death in asphyxia (48).However, Aly et al. suggested that IL-6 might have neurotrophic as well as neuroprotective, anti-inflammatory effects via inhibiting the synthesis of TNF-α and IL-1β (41).They found highly elevated IL-6 CSF to plasma ratios, and Martin-Ancel et al. also concluded that IL-6 appears to be primarily produced intrathecally following the ischemic brain injury while diffusion from the plasma is secondary' (48). 'Plasma IL-6 levels were elevated in severe compared to moderate asphyxia at 1 week and decreased in moderate, but not in severe asphyxia by 1 month. Intracellular levels of IL-6 in CD4+ cells peaked at 24 h in both patient groups and declined later.

However, no alterations were found in intracellular cytokine levels or cellular prevalence data between the two study groups, suggesting that CSF levels of IL-6 might be of more importance with regard to its deleterious effects' (44).

Cytokine pattern variation secondary to induced hypothermia post perinatal asphyxia injury: 'Systematic reduction of cerebral temperature with TH affects multiple phases of the hypoxic-ischemic injury. For every 1 °C decrease in body temperature, cerebral metabolic rate decreases by 6% to 7%, reducing cerebral energy demands (49). Additionally, TH modulates the primary phase of injury by decreasing the release of excitatory amino acids and the synthesis of nitrous oxide to reduce excitotoxicity (49). In the latent and secondary phases, TH blunts the release of reactive oxygen species triggered by reperfusion. TH suppresses inflammation, decreases expression of pro-apoptotic mediators, and decreases cytotoxic edema by preserving the blood-brain barrier (49). Hypothermia at least partially blocks several damaging reactions, by production of anti-inflammatory molecules like IL-10, TNF-α, and NO by activated microglia, the activation of NF-κB, the mRNA expression of the anti-inflammatory cytokine IL-10' (49).

'IL-6 plays a key role in stimulating or inhibiting steps in the cytokine cascade. Serum IL-6 levels were significantly lower in the hypothermia group at 6 h of age suggesting that hypothermia may immediately prevent the early rise of IL-6 following asphyxia. Moreover, the separate analysis of the cases of perinatal asphyxia who received therapeutic hypothermia revealed a significant negative correlation between IL-6 levels at 6 h of age and the duration of hypothermia, suggesting a 'dose dependent' reducing effect of hypothermia on the early rise of IL-6 serum levels. This provide further evidence that one mechanism by which hypothermia exerts its neuroprotective effect might be the suppression of the hypoxia induced inflammatory response and down grading of cytokines at cellular level—particularly by reducing the early rise of IL-6 levels' (50).

Neurodevelopmental assessment tools:

At discharge	**Hammersmith Neonatal neurological examination (HNNE)**
At 3.5 months	**Hammersmith Infant neurological examination (HINE)**

Hammersmith neonatal neurological examination (HNNE) (51-55):

HNNE is an internationally validated objective method, used for the neurological assessment of term and preterm infants. It is a simple and scorable method designed for evaluating neurological examination among preterm and term neonates. It encompasses 34 items

assessing 6 categories qualitatively and quantitatively (tone, tone pattern, reflexes, spontaneous movements, abnormal signs and behavioural items).

Its scoring is done in 2 ways:

i) Raw score

ii) Optimality score

Raw score: Each of the 34 items was scored on proforma given by Ricci et al (for assessing HNNE) by circling the most appropriate column (labeled as 1 to 5 and scored accordingly as 1 to 5 respectively). If an item fall between 2 columns it was given appropriate half score in between those two points. These scores are defined as raw scores for different items

Optimality score: Optimality score is based on the frequency distribution of the scores in the normal population, defining as optimal all the scores found in atleast 90% of a cohort of normal healthy population.

Optimality score in HNNE: Optimality score in HNNE is a quantitative assessment of neonatal neurological status based on neonatal neuro-behavioural examination which can be used to compare the neurological differences between preterm/at risk and term neonates. Optimality score for individual item of HNNE proforma is calculated based on frequency distribution of the raw scores in normal population and total optimality score is calculated by adding the optimality score of all 34items. For a given item, most frequently observed findings or findings seen in ≥90% of population or falling more than 10th centile is defined as optimal. Any item seen in less than 5%population or falling less than 5th centile is regarded as suboptimal. Items seen between 5-10% of population are classed as borderline.

Table 2.4: Different types of optimality scoring in Hammersmith neonatal neurological examination (HNNE)

HNNE OPTIMALITY SCORING	
Optimality scoring for individual items	- The raw score for any item falling above 10^{th} centile was given a score of 1 - Between 5^{th} to 10^{th} centile score is given 0.5 - Below 5^{th} centile score given is zero
Compound optimality score	Sum total of optimality score of the individual items in the category
Total optimality score	Sum of optimality score of all 34 items

Table 2.5: Compound and total optimality scoring in HNNE

<table>
<tr><th colspan="4">HNNE optimality score assessment (compound and total)</th></tr>
<tr><td rowspan="2">Compound optimality score</td><td>Tone:
9-10 = optimal
<9 = suboptimal</td><td>Reflexes:
5-6 = optimal
<5 = suboptimal</td><td>Abnormal signs
3 = optimal
<3 = suboptimal</td></tr>
<tr><td>Tone patterns:
5 = optimal
<5 = suboptimal</td><td>Movements:
3 = optimal
<3 = suboptimal</td><td>Behavioural items
6-7 = optimal
< 6 = suboptimal</td></tr>
<tr><td>Total neurologic optimality score</td><td colspan="3">30.5 to 34 = optimal
<30.5 = suboptimal*
*The presence of suboptimal global score does not necessarily means that an infant is neurologically abnormal but only identifies an infant who needs to be reassessed</td></tr>
</table>

Neurodevelopmental assessment on follow-up using HINE:

Hammersmith Infant neurological examination (HINE): 'Hammersmith Infant Neurological Examination (HINE) is an internationally validated objective method, used for the neurological assessment of term and preterm infants and their neurological impairment. It is a simple and scorable method for assessing infants between 2 to 24 months of age including items under 5 categories (cranial nerve function, posture, tone, movements and reflexes)' (56-60).

Optimality score: Optimality score is based on the frequency distribution of the scores in the normal population, defining as optimal all the scores found in atleast 90% of a cohort of normal healthy population.

Optimality score in HINE: 'The optimality score is obtained by calculating the distribution of frequency of the scores in the normal population, defining as optimal all the scores found to be inatleast 90% of the cohort. The overall score ranges from minimum 0 to maximum 78'.

Table 2.6: HINE optimality scoring at various ages in infancy and their interpretation as per HINE (90)

Age	Score
3 months	≥67 = optimal < 67 = suboptimal
6 months	≥ 70 = optimal <70 = suboptimal
9 months	≥ 73 = optimal <73 = suboptimal
12 months	≥ 73 = optimal < 73 = suboptimal
A progressive increase of score was observed until 12 months. 50% of the infants gain an optimality score of 73 or more by 12 months of age. All infants with mild disability (on BSID II done at 24 months corrected age) scored in the range of 40-66 at 3 months of age. In this group of infants, a progressive improvement of score of 3 months onwards was observed, but with minimal changing from 9 months. Optimal score in this age group to predict neuro-disability at later age needs to be decreased to <67. *HINE global score was correlated with the outcome with better correlation for the results at 9 and 12 months and for very preterm infants. HINE performed at 3 months shows lowest power of prediction with some items assessing sitting posture and defensive reactions could be considered less sensitive when assuming infants at 3 months of age. However infants with global score of ≤ 56 and ≤65 at 3 months and 12 months had a high probability of developing cerebral palsy with scores <40 reported.* ***For the purpose of this study optimal score at 3-3.5 months age will be taken as 67 or more***	

Hammersmith Infant Neurological examination (HINE) and its predicting probability of neurodevelopment outcome in infancy

'The HINE was used for the assessment of all infants enrolled in this study. This is a simple and scorable method for assessing infants between 2 and 24 months of age'.

'There are three parts to the HINE: a neurological examination (which is scored), developmental milestones and behaviour (which are not scored). The scorable neurological examination is comprised of 26 items divided into 5 domains, assessing cranial nerve function, posture, quality and quantity of movements, muscle tone, and reflexes & reactions.

Each item is scored individually (0, 1, 2 or 3). The maximum score for any one item is a score of 3 and the minimum is a score of 0. A subscore can be given for each section and the overall global score can be calculated by summing up all 26 items (range: 0–78), with higher scores indicating better neurological performance. The maximum global score is 78. An optimality score is obtained by calculating the distribution of the frequency of the scores in the normal population, defining as optimal all the scores found in at least90% of the cohort. The overall score ranges from a minimum of 0 to a maximum of 78. At 9 or 12 months, the scores equal or above 73 are regarded as optimal, if below 73 as suboptimal; while at 3 and 6 months healthy term infants scored equal or above 67 and 70 (median) respectively (57, 57). In this retrospective study, we analysed files of patients investigated by the HINE at3, 6, 9 and 12 months (corrected for prematurity) with the score of single subsections of items and a global optimality score for each period, according to the clinical protocol routinely performed in our NICU. Global cut-off scores were used as previously reported' (58-60).

'The HINE has good sensitivity and high predictive value for risk of cerebral palsy in high risk populations under 5 months. A HINE score < 57 at 3 months 96% predictive of cerebral palsy (sensitivity 96%; specificity 87%) (60).Over 5 months age corrected for prematurity it has 90% predictive accuracy for detecting the risk of cerebral palsy (61, 62). It provides objective information about likely motor severity and distribution of cerebral palsy (60). Scores below 40 predict non-ambulant cerebral palsy. It provides information on other aspects of neurological function other than motor. It has good inter-observer reliability for all levels of clinical experience(62-64).Previously HINE was used to to describe the neuro-motor development of infants with CP during the first year of age and to differentiate infants with diplegia from those with quadriplegia by a lower scoring of the latter in the subsections tone and posture. Infants with normal development shows higher global and subsections scores at all ages than both those with mild disability, with the exception of posture at 3 months, and with CP (when assessed with BSID II). A progressive increase of motor development is observed during the first year of from birth. This last result is quite different from the findings reported by Haataja et al who also showed, in low-risk term born infants, a progressive improvement before 7 months of age, but with little changes on the scores obtained at 9 and 12 months. In subsection scores, infants with normal development scored significantly better in all subsections than both those with CP and mild disability, even if the latter and N scored similarly for "posture" at 3 months; this data could reflect a similar early postural development in infants, with normal or quite normal motor outcome, who attended

an NICU. Infants with mild disability showed higher scores than those with CP for all subsections except for "cranial nerve" at 3 and 6 months, making this subsection less sensitive than others in differentiating these two populations of infants' (60).

'The HINE global score was correlated with the outcome, with a better correlation for the results at 9 and 12 months and for very preterm infants. It is not surprising that the findings of HINE performed at 3 months showed the lowest power of prediction. In fact, this tool was designed for and validated in older infants and some of the items assessing sitting posture and defensive reactions could be considered less sensitive when assessing infants at 3 months' (61).

'However infants with a global score <56 and <65 had a high probability to develop a CP, at 3 months and 12 months respectively, with scores <40 were reported in CP only. Previous researches reported cut-off scores similar to those taken in present study (58, 60, 61). Considering the different types of CP, the HINE scores could be used to identify only those infants with severe CP, whereas in infants with hemiplegia, due to an overlap of scores with those with normal and mild disability, the sensitivity is very low'.

'Other neurological tools have been used to predict the neurological outcome in high risk infants; mainly, in the first months of life the use of general movements showed a higher sensitivity and specificity of 98% and 94% respectively at 3 months of age for the development of CP' (65).

'At older ages, the Alberta Infant Motor Scale showed at 4 and 8 months a good sensitivity and specificity (77% and 82% at 4 and 86%and 93% at 8 months respectively) to predict abnormal outcome at 18 months in risk newborns (66**).** However, these tools showed a lower prediction when assessing infants at low risk' (67, 68).

'Compared to these tools, the HINE showed the advantage to predict the motor outcome in low and high risk infants with a good power prediction at different ages. HINE is a helpful tool in the process of early diagnosis of infants at low and high neurological risk, with a good predictive value. The use of the cut-off scores at different ages allows us to follow longitudinally high risk infants during the first age from birth to identify those infants in need of specific rehabilitation programme' (60).

'It is essential to follow all the neonates with moderate and severe asphyxia, especially those with stage 2 and 3 HIE. They should have a complete neurological assessment and early intervention, if needed during follow-up. A formal psychometric assessment at 18 months should be performed in them'.

Role of neuroimaging in assessment of perinatal brain injury secondary to HIE: (81)

'MRI is invaluable in assessing the neonatal brain following suspected perinatal injury. Good quality imaging requires adaptations to both the hardware and the sequences used for adults or older children. The perinatal and postnatal details often predict the pattern of lesions sustained and should be available to aid interpretation of the imaging findings. Perinatal lesions, the pattern of which can predict neurodevelopmental outcome, are at their most obvious on conventional imaging between 1and 2 weeks from birth. Very early imaging during the first week may be useful to make management decisions in ventilated neonates but brain abnormalities may still be subtle using conventional sequences. Diffusion-weighted imaging (DWI) is very useful for the early identification of ischaemic tissue in the neonatal brain but may underestimate the final extent of injury, particularly basal ganglia and thalamic lesions (BGT). MR imaging is an excellent predictor of outcome following perinatal brain injury and can therefore be used as a biomarker in interventional trials designed to reduce injury and improve neurodevelopmental outcome'.

'There has been speculation that treatment with hypothermia will delay the appearance of abnormalities on MRI. It is possible that with the routine use of hypothermia as a treatment strategy in clinical practice we will be in a better position to assess the effect of hypothermia on imaging appearances. With or without hypothermia the second week from delivery is the ideal time to detect the extent of acquired lesions on conventional imaging. In the recently published TOBY trial the presence of hypothermia did not influence the ability of a neonatal MRI (median 8 days) to predict neurodevelopmental outcome' (82).

'Following a hypoxic-ischaemic brain insult proton spectroscopy (MR spectroscopy) may demonstrate an increased lactate peak; this is useful to confirm the presence of a significant tissue injury, particularly in early examinations before conventional imaging becomes overtly abnormal. Proton spectroscopy has also been used to predict outcome in the research environment and has a good sensitivity and specificity for poor outcomes in neonates with hypoxic-ischaemic encephalopathy' (HIE) (83).

Patterns of injury on MRI brain and prediction of outcome

'In neonates with a global hypoxic-ischaemic insult, lesions are usually detected within the BGT with abnormal signal intensity (SI) in the intervening posterior limb of the internal capsule (PLIC). Abnormal SI within the PLIC is an excellent predictor of abnormal motor

outcome in term infants with HIE (84). BGT lesions give rise to motor impairment in the form of cerebral palsy. The severity of the BGT lesions dictates the severity and nature of the cerebral palsy (28). Lesions within the brainstem are usually found in those neonates with the most severe form of BGT injury and are often associated with early death (85). In those neonates that survive, persisting feeding difficulties and a necessity for gastrostomy are common. In the presence of severe BGT injury there are usually secondary WM changes with subsequent atrophy, poor head growth with the development of a secondary microcephaly and cognitive impairment (86). In approximately 50% of neonates with BGT lesions there will be more extensive WM abnormalities seen on the initial scan consistent with a primary injury. The motor outcome for these children is still dictated by the BGT lesions but the more severe WM involvement may exacerbate any cognitive deficit and results in very poor head growth (86). In some neonates who present with what is thought to be HIE there is no BGT involvement but only WM lesions. These may be haemorrhagic giving rise to tissue atrophy, poor head growth and the development of a secondary microcephaly and are associated with later cognitive impairment. The more severe the WM lesions the worse the cognitive outcome and the greater the likelihood of some motor impairment' (87, 88). 'In neonates who have a term perinatally acquired WM injury, follow-up imaging may be indistinguishable from what would normally be called periventricular leucomalacia (PVL), an injury thought specific to the more preterm brain. Without knowledge of the clinical neonatal history, the injury may therefore be wrongly attributed to antenatal damage at an earlier gestation. However in an infant with signs of PVL, on imaging and/or clinically, who was born at term it is probably only reasonable to implicate perinatal events if there were neonatal symptoms such as encephalopathy or seizures. Focal infarction usually involves the territory of the middle cerebral artery, the left side being more commonly affected than the right. The outcome following an MCA infarct depends on the extent and sites involved. If there are abnormalities within the parenchyma, BGT and PLIC then the child is likely to develop a later hemiplegia' (89).

Long term outcome in perinatal asphyxia:

'Neonates who survive severe HIE, the sequelae include mental retardation, epilepsy and cerebral palsy. Cerebral palsy can be in the form of hemiplegia, paraplegia or quadriplegia. These infants need to be referred to specialized clinics capable of providing coordinated comprehensive follow-up care'.

‘The incidence of long term complications depends upon the severity of HIE. Upto 80% of neonates with stage 3 HIE die whereas rest 20% have neurological sequelae. Upto 80% of the neonates who survive severe HIE develop serious complications, 10-20% develop moderately serious disabilities, and upto 10% are normal. Among newborns who survive moderately severe HIE 30-50% may suffer from serious long term complications, and 10-20% with minor neurological morbidities. Neonates with mild HIE tend to be free from death or any neurological sequelae’ (69, 70).

‘Whole body hypothermia leads to combined end point of death or an IQ of <70 at 6 to 7 years of age to be lower among neonates undergoing whole body hypothermia (47%) than those undergoing usual care’ (62%) (71).

‘Prognostic pointers/markers of mortality and neurological morbidity after perinatal hypoxic-ischemic insult:

1. **Clinical markers**- seizure, requirement and duration of invasive ventilation, shock, higher mental function and consciousness and alertness level, time of onset of sleep wake cycling (SWC in < 36 hours indicate good prognosis)

2. **Cord blood parameters**– especially pH, base deficit and lactate. Presence of metabolic acidosis indicate relatively long standing asphyxia (minutes to hours), while presence of respiratory acidosis in the absence of metabolic acidosis indicate acute asphyxia (minutes) as in cord prolapse, acute abruption of placenta etc. Cord blood gas abnormalities (pH <7 and base deficit of ≥ 16 mmol/l) have a good correlation with short term (mortality, HIE, IVH or PVL) and long term adverse outcomes (cerebral palsy).
3. **Resuscitation measures requirement initial clinical assessment** (low extended Apgar score at 20 minutes or more, time to establish spontaneous respiration)
4. **Neonatal neurological examination** (labeling moderate to severe HIE) – as per Sarnat and Sarnat classification or Levene classification, Thompson scoring and how these scoring improves or worsens over time with or without intervention.
5. **Brain neuroimaging** - On USG hypoechoic areas can be seen in very severe cases (having large areas of infarction). It is also helpful in preterm neonates in detecting PVL and IVH during first week of life. MRI is the best imaging modality for determining prognosis in term neonates. Diffusion weighted MRI can detect abnormalities within 24 to 48 hours after birth whereas conventional MRI show

optimum results in later part of first week. An altered signal at the level of posterior limb of the internal capsule and abnormalities of the thalami and basal ganglia in term neonates and that of white and grey matter at term equivalent age in preterm neonates are strong predictors of subsequent risk of poor neurodevelopmental outcome (72).

6. **Other investigations** (EEG, aEEG, evoked potentials like BERA). The prognosis is likely to be poor, if EEG shows any of the following: long periods of inactivity (>10 seconds), brief period of bursts (< 6 seconds) with small amplitude bursts, inter-hemispheric asymmetry and asynchrony, isoelectric and low voltage (<5 microvolts). On aEEG wide fluctuations in the amplitude with the baseline voltage dropping to near zero, peak amplitude < 5 millivolts and seizure spikes denote poor prognosis.
   *of the multiple prognostic test available, aEEG in the first 6 hours showed maximum sensitivity and specificity (93% and 90%, respectively) (73).
7. **Molecular markers** of inflammation and tissue injury (raised CRP, interleukin levels in blood and CSF, raised markers of cardiac tissue (CK-MB) and renal tissue injury (urine output, NGAL, KIM-1 etc)'

Table 2.7: Review of articles assessing cytokines in perinatal asphyxia and its subsequent neurodevelopmental outcomes:

Indian literature (74)

Study/Author/Year	Objectives	Markers	Tool of assessment/Time of assessment	Population studied	Result	Place
'Inflammatory mediators as predictors of outcome in perinatal asphyxia' *Bharathi et al* *2014*	'To identify biomarkers for neurone injury and outcome in perinatal asphyxia'	'IL-6, IL-1b, IL-2, TNF-alpha in cord blood'	Baroda developmental score at 6 months	40 newborns with perinatal asphyxia and 40 normal newborns as control	'Cytokine levels (IL-6, IL-1b) were significantly higher in perinatal asphyxia' Significant negative correlation with developmental score at 6 months	JIPMER, India

Western literature (75-77)

Author/year	Case group	Control group	Markers	Time of assessment	Sensitivity	Specificity	PPV/NPV	Results	Place
Caroline E. Ahearne et al 2017	69 babies with evidence of asphyxia at birth		Cord blood IL-16	At birth sample taken; neurodevelopmental assessment with BSID at 36 to 42 months				'High cord blood IL-16 were associated with severely abnormal neuro-developmental outcomes at 3 years of age'	Ireland
Bajnok et al 2017	28 newborns with moderate to severe birth asphyxia requiring therapeutic hypothermia		IL-1b, IL-6, TNF-alpha, TGF-b	Between 3 to 6 hours, at 24 hours, at 72 hours, 1 week, and 1 month of life				'IL-1b at 6 hours can predict severity of insult, at 1 month level of TNF-alpha was higher in severe group, IL-6 value was high at 1 week but normalised by 1 month in moderate asphyxia, intracellular TGF-b levels begins to rise from 24 hours inwards in the moderate group'	Hungary
Boskabadi et al 2016	38 uninfected infants with perinatal	47 healthy infants	UL-1b, IL-6	At birth	IL-1b – 71% IL-6 80%	IL-1b – 1.89%, IL-6 –		'Simultaneous evaluation of of IL-Ib and IL6 can	Iran

	asphyxia					6.81%		improve the sensitivity of early diagnosis of perinatal asphyxia'	
Boskabadi et al 2010	37 uninfected infants with perinatal asphyxia	45 healthy infants	IL-6	At birth, 24 hours and 48 hours post delivery	94.5%	97.7%	97%/95%	'IL-6 serum is useful predictors for the outcomes of HIE and intensity of perinatal asphyxia'	Iran
					93.7%	81.5%			
					88.2%	96.9%	88.2%/96.9 %		
Boskabadi et al 2010	38 uninfected infants with perinatal asphyxia	41 healthy infants	IL-6	At birth, 24 and 48 hours post delivery	77%	38%	4.71%/4.78 %	'IL-1b serum is predictor of term neurological consequences and intensity of perinatal asphyxia'	Iran
					7.85%	78%			
Chiese et al 2003	50 uninfected infants with perinatal asphyxia	113 healthy infants	IL-6	At birth, 24 hours and 48 hours post delivery	80.9%	70.8%		'IL-6 assessment in umbilical cord is useful in early diagnosis of infants at high risk of brain damage and adverse consequences'	Italy
Paliwal et	50 infants with	100 healthy	IL-6	First and 3rd day				'IL-6 is increased	India

al 2014	perinatal asphyxia	infants		of life				after asphyxia at birth which is associated with severity of HIE and poor outcomes'	
Silveira et al 2003	15 infants with HIE	20 healthy infants	IL-6 in CSF	48 hour post birth				'IL-6 and TNF –α levels are more in term infants or HIE than with that in healthy infants'	Brazil
Oygur et al 1998	19 infants (14 with abnormal developme nt and 5 infant deaths)	11 healthy infants	IL-1b in plasma and CSF					'IL-1b is associated with CNS damage after hypoxia and can be useful predictor of HIE'	Turkey
El Farargy et al 2014	27 infants with evidence of HIE	25 healthy infants	IL-6	12 hours post birth				'The assessment of IL-6 levels may be useful in early diagnosis of perinatal asphyxia'	Egypt
Shang et al 2014	74 infants with HIE	74 healthy infants	IL-6					'High levels of IL-6, TNF-alpha and CRP was observed in neonates with HIE'	China
Skouteli et al	29 infants with perinatal	28 healthy	UL-1b and IL-	24 hrs, 3rd day, 7th day				'Increased levels of pro-	Greece

2001	asphyxia	infants	6 serum					inflammatory cytokines in neonates with asphyxia is indicative of future neurological disorders'	
Martin-Ancel et al 1997	5 infants with brain injury symptoms	14 healthy infants	IL-6	8 and 90 hours after delivery				'Increase in IL6 in CSF in perinatal asphyxia is associated with severe HIE, brain damage and neurological outcome'	Spain
YH Zhou et al 2004	50 infants with perinatal asphyxia	20 healthy infants	IL-6	1, 3 and 7 day of life				'IL-6 level increases in neonatal asphyxia'	China
Alsulaimani et al 2015	29 infants with HIE	55 healthy infants	IL-6	At birth, 24 hours and 72 hours of life				'Serum levels of IL-1, IL-6, TNF-alpha increases in neonates with HIE'	Saudi Arabia

Importance of cytokine assessment in perinatal asphyxia:

Cytokine cascade is responsible for inflammatory response leading to disruption of blood brain barrier and production of secondary insult in neonatal brain because of various cellular reactions in hypoxic-ischemic insult. So their quantitative pattern will tell about the future neurodevelopment outcome.

3. GAPS IN KNOWLEDGE AND RATIONALE

What is known till now?

1. There is rise in levels of all cytokines in acute perinatal hypoxic ischemic insult.
2. Sepsis also causes rise in levels of cytokines.
3. Cord blood cytokine has some correlation with long term neurodevelopmental outcome.
4. Therapeutic hypothermia causes decrease in release of these cytokines.

Gaps in knowledge:

1. Most of the previous studies correlating cytokines levels with neurodevelopmental outcome took serum samples at single time point (either cord blood or early neonatal period). No study followed cytokine pattern over a period of time to look for trend in different grades of asphyxia and how their value changes over a span of time with presence/absence of sepsis.
2. No study correlating cytokine pattern with neuroimaging (MRI grades of perinatal asphyxia injury).
3. Limited data on intact cord resuscitation present, its impact on cytokine pattern, asphyxial status and neurodevelopmental outcome is a field of interest yet to be fully explored.

What is new in this study?

1. We will try to correlate the cytokine pattern with neurological status monitored at various time interval in early infantile period with HNNE (in neonatal period) or HINE (beyond 2 months of age).
2. We will also assess the role of neonatal sepsis in modifying the cytokine pattern and clinical outcome
3. We will try to correlate the cytokine pattern with the grade and severity of neurological lesions seen on MRI brain.
4. Our study will be the first prospective cohort study correlating cytokine pattern with level of perinatal asphyxia in term newborns in Indian setting.

4. RESEARCH QUESTION

Is there any correlation between the cytokine levels (IL-6, IL-1 beta), CRP and in newborns having perinatal asphyxia with moderate to severe HIE and their neurodevelopmental outcome at 3½ months of age?

AIMS AND OBJECTIVES

Primary objective:

To correlate cytokine pattern with neurodevelopmental assessment at 3½ months of age(using Hammersmith Infant Neurological Examination – HINE).

Secondary objectives:

1. To find association of cytokine pattern with in-hospital neonatal mortality.
2. To find association of cytokine levels with degree of perinatal asphyxia
3. To correlate cytokine pattern with neurodevelopment status at discharge (using Hammersmith neonatal neurological examination - HNNE).
4. Role of neonatal sepsis in modifying the cytokine pattern and the clinical outcome.
5. Correlation between cytokine pattern and neurological lesions seen on MRI brain done between day 5 to 21`of life.

5. MATERIALS AND METHODS

5.1: Methodology

Study Design: A prospective cohort study.

Study Setting: Department of Neonatology & Department of Obs. & Gynaecology, All India Institute of Medical Sciences, Jodhpur (Rajasthan), India.

Study Period: January 2021 to December 2022.

Screening population of interest in labour room: Inborn term newborns (≥37 week period of gestation) with moderate to severe perinatal asphyxia as defined by NNPD Network (APGAR of ≤6 and ≤3at 1 minute of life respectively) (78) were screened and their cord blood sample were taken and stored for cytokines and cord blood gas analysis was done.

Newborns were further followed closely for any evidence of moderate to severe hypoxic ischemic encephalopathy (HIE) to ascertain if baby fulfils the eligibility criteria as follows:

Inclusion Criteria: Inborn term newborns (≥37 week period of gestation) with perinatal asphyxia with evidence of moderate to severe HIE as per the following criteria (79, 80):

Table 5.1: Biochemical Criteria for Neonates with Eligibility for Inclusion in moderate to severe HIE

Biochemical Criteria	Blood gas within 1st hour of life o (Cord, ABG, CBG, VBG) pH ≤ 7 **OR** base deficit ≥ 16
	Additional Criteria o If NO blood gas result available o **OR** o 1st hour gas pH is between 7.01-7.15 o **OR** o Base deficit 10-16 o **AND both of the following** o 10 min Apgar ≤ 5 **OR** assisted ventilation at birth continued for ≥ 10 minutes ▪ Not limited to intubation and mechanical ventilation, may include CPAP o An acute perinatal event: ▪ Late and/or variable decelerations, ▪ Cord prolapse/rupture ▪ Uterine rupture ▪ Maternal trauma, hemorrhage, cardiorespiratory arrest ▪ Shoulder dystocia ▪ Nuchal cord ▪ Other event leading to occurrence of hypoxia

- Once these criteria were met, all infants underwent a standardized neurologic examination.
- Infants were candidates for the study when encephalopathy or seizures were present.
- Encephalopathy was defined as the presence of one or more signs in at least three of the following six categories: level of consciousness, spontaneous activity, posture, tone, primitive reflexes (suck or Moro), and autonomic nervous system (pupils, heart rate, or respiration).
- The number of moderate or severe signs determined the extent of encephalopathy as defined by modified Sarnat staging; if signs of moderate and severe encephalopathy were found to be equally distributed, then the labeling was based upon level of consciousness.

Table 5.2: Criteria for defining moderate and severe encephalopathy as per modified Sarnat staging

Category	**Moderate encephalopathy**	**Severe encephalopathy**
Level of consciousness	Lethargic	Stupor or coma
Spontaneous activity	Decreased activity	No activity
Posture	Distal flexion, complete extension	Decerebrate
Tone	Hypotonia (focal or general)	Flaccid
Primitive reflexes Suck Moro	 Weak Incomplete	 Absent Absent
Autonomic system Pupils Heart rate Respiration	 Constricted Bradycardia Periodic breathing	 Deviated, dilated or non reactive to light Variable Apnea

Exclusion Criteria (any of the under mentioned):

1. Major congenital malformations/chromosomal anomalies.
2. Outborn neonate

5.2 PLAN OF STUDY

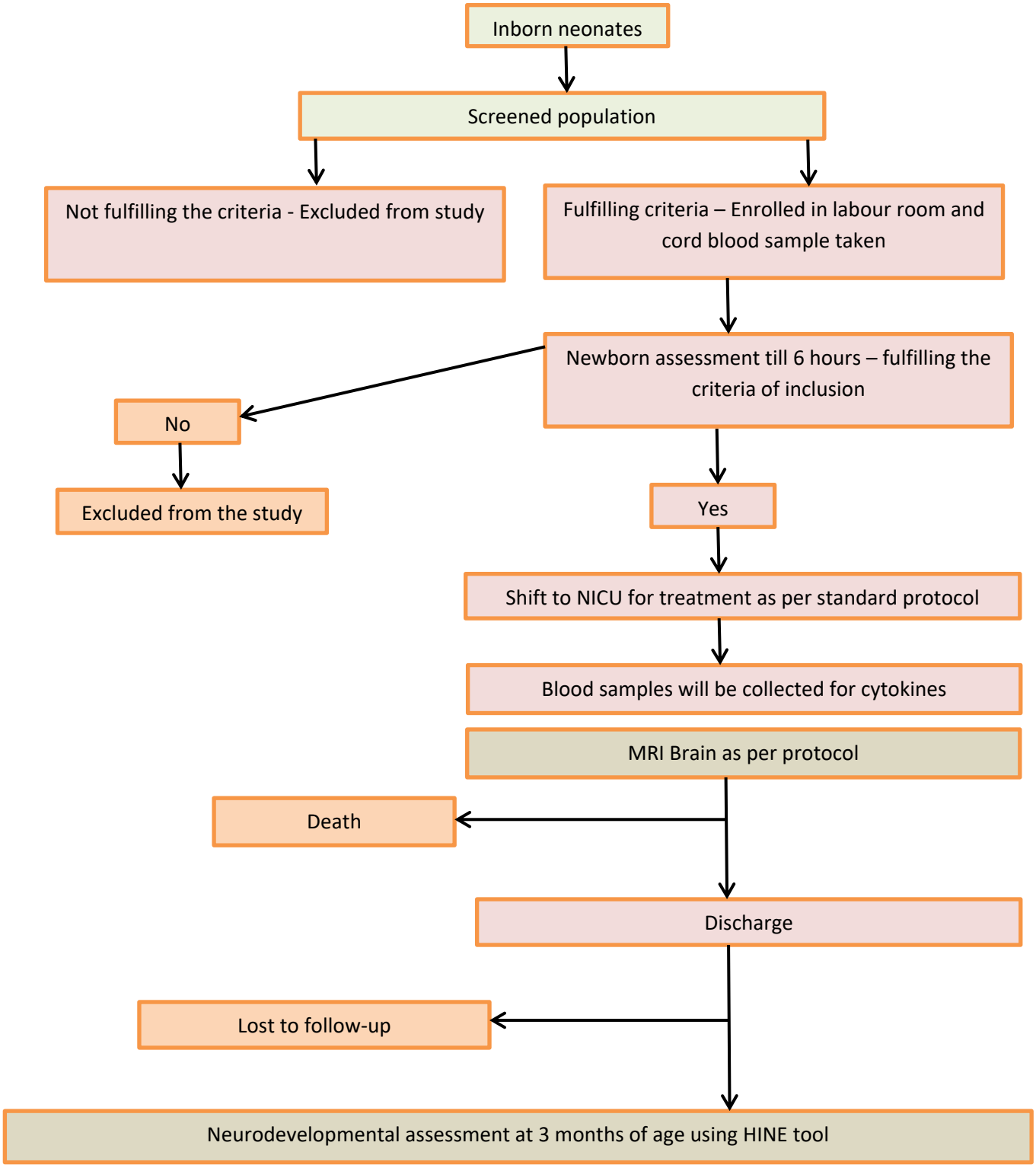


Figure 5.1: Flow diagram of the proposed protocol for this study

Enrolment of the test sample: Newborns were enrolled post screening and consent from the parents/guardian. Detailed maternal history of any infection/antepartum asphyxia was noted. After delivery Apgar was assessed at 1 minute of life. If it was ≤6, cord sample was taken and stored. Newborn was assessed over next 6 hours for evidence of moderate to severe birth asphyxia. Newborns showing evidence of moderate to severe birth asphyxia were enrolled in the study and were shifted to NICU for further intervention. Blood samples for cytokines assessment were taken at various time intervals as planned till 72 hours of life (details mentioned below) and stored. Newborns enrolled in study were managed as per the standard protocol and this study by no means affected their management as per the standard protocol. Hammersmith neonatal neurological examination (HNNE) was performed at the time of discharge. These newborns were followed in high risk clinic and newborn's neurodevelopmental status was re-assessed at 3½ months of age using Hammersmith infant neurological examination (HINE) tool. For every newborn enrolled in the study with evidence of moderate to severe perinatal asphyxia, MRI was done between day 5 and 21 as per the current protocol.

Enrolment of controls: From the inborn neonates, healthy controls without any evidence of birth asphyxia and matched for gestational age, weight and sex were enrolled in the study after obtaining written informed consent from the parent/guardian. Blood samples for cytokines assessment were taken as planned in the protocol. Neurodevelopmental assessment was done at 3½ months.

Samples taken:

Time point of sampling**	Investigations done	Blood volume required
Cord blood*	IL-6, IL-1b	1 ml
Between 2 to 6 hours of life (before starting therapeutic hypothermia)	IL-6, IL-1b	1 ml
At 24 +/- 2 hours of life	IL-6, IL-1b	1 ml
At 72 +/-2 hours of life	IL-6, IL-1b	1 ml
*cord blood sampling during labour room primary enrolment was as per the standard departmental protocol of AIIMS Jodhpur and there was no deviation or compromise in treatment or resuscitation due to this research. **the timing of these samplings was merged with the timings of the routine clinical sampling to avoid unnecessary pricks to the newborn. To avoid additional pricks, samples were drawn from arterial line, if present.		

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Neurodevelopmental assessment
At 3½ month of age using Hammersmith Infant Neurological examination (HINE) – done by thesis candidate(trained by guide to perform Hammersmith Neurodevelopmental assessment using HNNE and HINE at various time points as planned in study)

Sample collection: Samples were collected in one plain vial (1 ml) at planned intervals.

Sample storage: Samples were stored in deep freezer post centrifugation and separation of serum/plasma at -80 degree Celsius in department of Biochemistry at AIIMS, Jodhpur.

Sample processing: Samples were frozen at -80^{o}C and IL-6 was assessed later using chemiluminiscence technique based on ELISA principle and IL-1β samples were analysed using kits based on flow cytometry technique.

Sepsis criteria: Neonates with maternal risk factors or clinical features suggestive of sepsis were worked up for sepsis, which included blood culture, micro ESR, CRP/PCT, immature/total neutrophil ratio and total leucocyte count (TLC). A neonate was considered to have sepsis if blood culture was positive or had clinical parameters suggestive of sepsis along with two positive screening tests (micro ESR >15 mm/h, CRP >10 mg/dl, immature/total neutrophil ratio >0.2, TLC <5000).

Follow up visits: Enrolled babies were reviewed at discharge and 3½ months of age. Detailed neurodevelopmental assessment was done using Hammersmith assessment tool (HNNE & HINE at discharge and 3½month respectively).

MRI brain: All the neonates with evidence of moderate to severe HIE enrolled in study underwent MRI brain imaging as planned between initial 5 to 21 days.

5.3 The following outcomes were studied:

Primary outcome: To find correlation between IL-6 at 24 hours of age with neurodevelopmental assessment done at 3½ months of age using Hammersmith Infant Neurological Examination – HINE (HINE assessment was done by thesis candidate for every infant enrolled in study).

Secondary outcome:

1. To find mean difference in cytokine levels between infants who died as compared to infants who survived among infants with moderate to severe perinatal asphyxia.
2. To find mean difference between cytokine levels with degree of perinatal asphyxia.
3. To find correlation coefficient between cord blood cytokines (IL-6, IL-1β) with neurodevelopmental assessment done at discharge using HNNE.

4. To find correlation coefficient between cord blood cytokine (IL-6, IL-1β) with neurodevelopmental status done at 3½ month of age using HINE.

5. To find correlation coefficient between cytokine levels (IL-6, IL-1β) at 24 hours and neurodevelopmental assessment at discharge using HNNE.

6. To find correlation coefficient between cytokine levels (IL-6, IL-1β) at 2-6 hours, 24+/-2 hours and 72+/-2 hours and neurodevelopmental assessment done at discharge and 3½ months using HNNE and HINE respectively.

7. To find the role of neonatal sepsis in modifying the cytokine pattern and the clinical outcome

8. To find correlation coefficient between cytokine pattern and neurological lesions seen on MRI brain in newborns with perinatal asphyxia with moderate to severe HIE.

9. Receiver operating characteristic (ROC) curve will be plotted to determine sensitivity and specificity of interleukin (IL) levels in predicting adverse outcome.

5.4 Sample size calculation:

Assuming a correlation coefficient (r) between cytokine (IL-6) levels with optimality score of Hammersmith infant neurological examination (HINE) at 6 months to be -0.4 [74] with alpha error of 5% and power of 80%, the sample size of 47 was planned initially. Assuming 15% in hospital mortality with 15% lost to follow up rate, the total calculated test sample size was 67.

Total control sample of 33 were enrolled in the study as planned.

Being time driven study, convenient sample size was taken as it was difficult to meet required number of samples in the given time period and all eligible newborns were enrolled as cases and all enrolled cases were followed till 31/2 months of age.

6. STATISTICAL ANALYSIS

Basic demographics were expressed as percentages for categorical variables and continuous variables as mean (SD) for normally distributed data on the Shapiro Wilk test and as median (1st, 3rd quartile) for skewed distributions. Categorical variables were compared between groups by chi-square test or Fisher's exact test as applicable. Normally distributed continuous variables were compared by Students t-test and skewed data by Mann-Whitney U test. Correlation coefficient were calculated with either Pearson correlation coefficient or Spearman correlation coefficient as per distribution of data. Subsequently receiver operator curve (ROC) was made and a cutoff was derived which gives best combination of sensitivity and specificity. Statistical significance was set at $P < 0{\cdot}05$ with two-tailed testing. Whole data was analyzed using SPSS version 23.0 statistical software for Windows.

7. ETHICAL JUSTIFICATION

This study was undertaken only after obtaining ethical clearance from the Institutional Ethics Committee.

Subjects were enrolled only with the written informed consent from legally authorized representative (LAR) (consent form enclosed).

They were provided detailed information about the nature and purpose of study prior to enrollment.

Confidentiality of the records was maintained.

Data was collected as per prior set case record proforma.

Participation in, or withdrawal from this study had no bearing on the treatment being offered to the child.

8. RESULTS

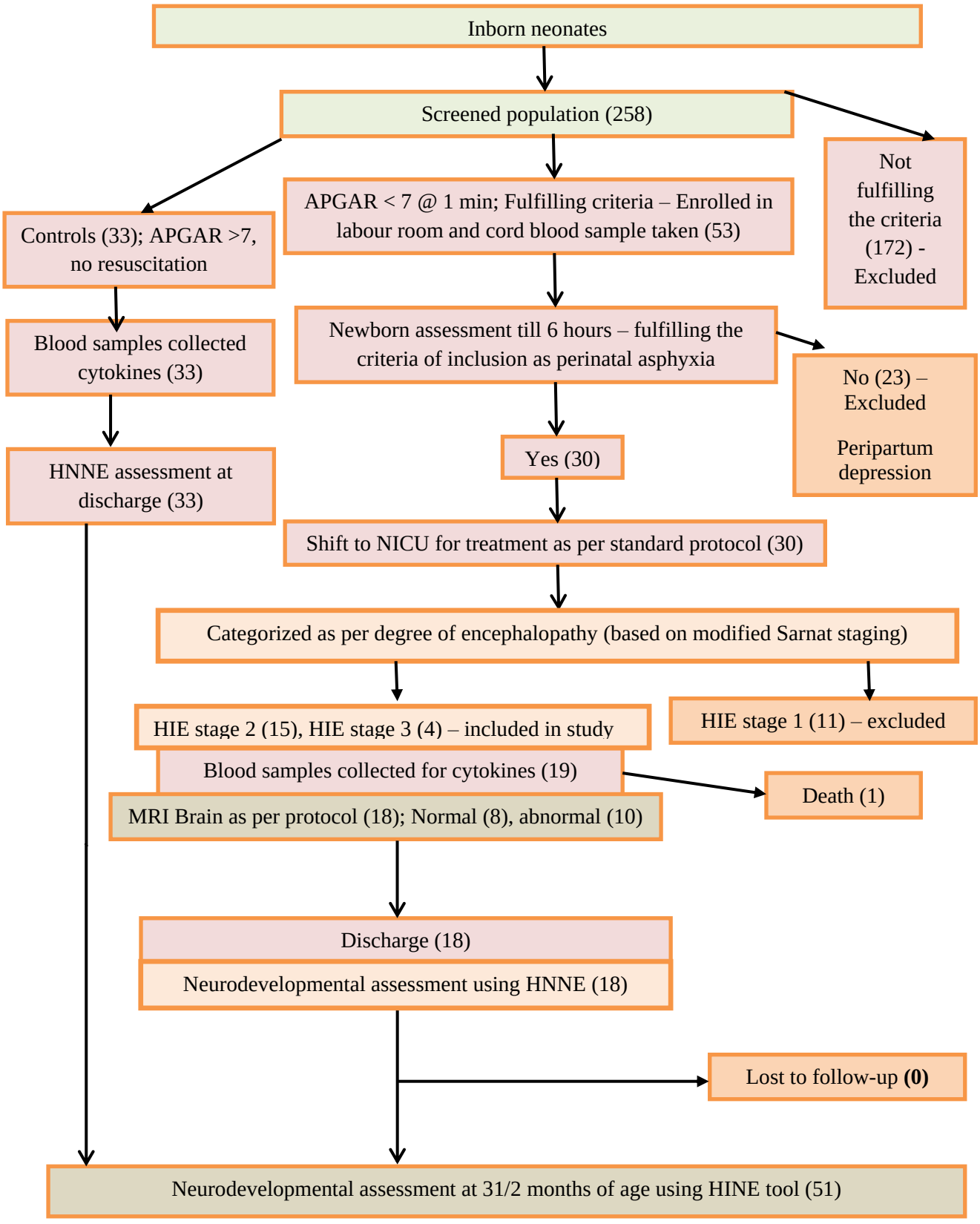


Figure 8.1: Flow of participants in this study

Among the 19 infants enrolled in the asphyxia group, 1 infant died during NICU stay on day 2 of life. Remaining 18 neonates in the asphyxia group and all 33 of the normal infants were followed till $3_{1/2}$ months of age with no loss to follow-up.

The interleukins were analysed at predefined time intervals for all infants from both the group. Neurodevelopmental follow-up of 18 infants in asphyxia group and 33 infants in control group was done at 3.5 months of age using HINE (Hammersmith infant neurodevelopmental examination).

8.1 BASELINE CHARACTERISTICS

Table 8.1: Demographic details and maternal baseline parameters

Parameter	Asphyxia group (n=19)	Normal neonates (n=33)	p value
Age of mother (years)	28.5 +/- 7.2	26.0 +/- 3.9	0.112
Booked and supervised	11 (57.9)	19 (57.6)	1.0
Gravid status (Primigravida)	12 (63.2)	12 (36.4)	0.08
Socioeconomic status of family Lower class	4 (21.1)	7 (21.2)	1.000
Fetus number Twins	5 (26.3)	0 (0)	0.004*
Past obstetric history			
Still born	2 (10.5)	0 (0)	0.129
Abortion	3 (15.8)	8 (24.2)	0.726
Maternal medical disorders			
Thyroid disorder	3 (15.8)	3 (9.1)	0.656
Chronic hypertension	2 (10.5)	0 (0)	0.129
Diabetes	1 (5.3)	0 (0)	0.365
Others	2 (10.5)	2 (6.1)	0.617
Obstetrics issues			
Anemia	6 (31.6)	1 (3)	0.07
GDM	2 (10.5)	4 (12.1)	1.0
Gestational hypertension	3 (15.8)	3 (9.1)	0.656
Respiratory illness (ARDS etc.)	2 (10.5)	0 (0)	0.129
Rh negative pregnancy	2 (10.5)	1 (3)	0.546
Liver disease (IHCP)	0 (0)	3 (9.1)	0.291
*Data are represented as n (%), Mean+/-S.D. n (%) denotes the number of total subjects with their percentage in brackets. Fischer exact test is applied for significance. P-value <0.05 considered significant and denoted with **			

The baseline parameters revealed that there was no statistically significant difference between the two groups in terms of maternal age, parity, socioeconomic status, ANC check-up, previous still-born/abortions, and gestational age.

Table 8.2: Maternal obstetrics parameters comparison between asphyxia and healthy control infants

Parameter	Asphyxia group (n=19)	Normal neonates (n=33)	p value
Maternal Hb (g/dL)	11 +/- 1.5	11.8 +/- 1.3	0.056
Maternal TLC (X 10^3/micro L)	9.51 +/- 1.38	9.79 +/- 4.08	0.687
Maternal TSH (mIU/L)	1.93 +/- 0.55	2.2 +/- 1.0	0.350
Weight gain during pregnancy (kg)	11 +/- 1.3	11.09 +/- 1.20	0.096
Antenatal progesterone given	0 (0)	1 (3)	1.000
Antenatal steroids given	1 (5.3)	1 (3)	1.000
Antenatal $MgSO_4$ given	2 (10.5)	0 (0)	0.129
Antepartum hemorrhage	1 (5.3)	0 (0)	0.365
Data are represented as n (%), Mean+/-S.D. n (%) denotes the number of total subjects with their percentage in brackets.			

Table 8.3: Peripartum characteristics of study population

Parameter	Asphyxia group (n=19)	Normal neonates (n=33)	p-value
Induction done	6 (31.6)	10 (30.3)	1.00
A**ugmentation done**	6 (31.6)	10 (30.3)	1.00
Meconium-stained liquor	4 (21.1)	1 (3)	0.054
Other intrapartum parameters			
Maternal fever	1 (5.3)	0 (0)	0.365
Foul smelling liquor	0	0	1.00
Clinical chorioamnionitis	1 (5.3)	0 (0)	0.365
Antenatal risk factor for infection	1 (5.3)	0 (0)	0.365
CTG decelerations	34	11	0.002*
CTG decelerations type			
No	4 (21.1)	24 (72.7)	
Early	2 (10.5)	9 (27.3)	
Variable	7 (36.8)	0 (0)	
Late	6 (31.6)	0 (0)	
Mode of delivery			
Spontaneous vaginal	1 (5.3)	13 (39.4)	
Induced vaginal	0 (0)	8 (24.2)	
Vaginal operative (forceps, vacuum	5 (26.3)	0 (0)	

assisted) Elective LSCS Emg. LSCS	0 (0) 13 (68.4)	8 (24.2) 4 (12.1)	
LSCS anaesthesia used			
Spinal	9 (47.4)	12 (36.4)	0.37
General	4 (21.1)	0	0.006*
Opioids before clamping	5 (26.3%)	0	0.004*
*Data are represented as n (%), Mean+/-S.D. n (%) denotes the number of total subjects with their percentage in brackets. P-value <0.05 considered significant and denoted with **			

Maternal hematological & biochemical parameters, medical and obstetric history were also not statistically different among two groups. 68.4% of the fetus in asphyxia group had variable or late decelerations on CTG. 4 neonates (21.1%) in the asphyxia group had meconium stained liquor as compared to 1 in healthy control group. 1 case in asphyxia group had clinical evidence of chorioamnionitis. Asphyxial group had higher number of twin deliveries and emergency LSCS and 4 neonates (21.1%) were delivered under general anesthesia in asphyxia group because of need of urgent intervention whereas 5 neonates (26.3%) were born to mother already on opioid medication (sedation and analgesia) because of maternal indication.

Table 8.4: Neonatal characteristics of study population

Parameter	Asphyxia group (n=19)	Normal neonates (n=33)	p value
Birth weight (kg)	2.83 +/- 0.43	2.98 +/- 0.38	0.234
Length (cm)	48.7+/- 2.1	49.3 +/- 1.53	0.263
OFC (cm)	33.5 +/-1.5	34.2 +/- 0.98	0.071
Gestational age (days)	268.5 +/- 10.3	270 +/- 7.3	0.586
Gender (male)	12 (63.2)	16 (48.5)	0.391
SGA	4 (21.1)	7 (21.2)	1.00
Cord TSH (mIU/L)	10.1 (7.35, 29.2)	5.9 (4.7, 10.3)	0.012*
Data are represented as n (%), Mean+/-S.D. n (%) denotes the number of total subjects with their percentage in brackets; TSH values represented as median, interquartile: M (Q1, Q3). Significance calculated by student t-test and Mann-Whitney U test for parametric and non-parametric data respectively. *p-value <0.05 considered significant and denoted with **			

Infants born in both the group had statistically insignificant difference in gender, birth weight, length and OFC. Cord TSH levels were significantly higher in asphyxia group as compared to controls.

Table 8.5: Resuscitation parameters of the study population

Parameter	Asphyxia group (n = 19)	Normal neonates (n =33)	p-value
1 minute			
APGAR score	2.4 +/- 1.54	8.2 +/- 0.48	0.0001*
Expanded APGAR score	3.73 +/- 0.8	7 +/- 0	0.0001*
Combined APGAR score	6.2 +/- 1.87	15.2 +/- 0.48	0.0001*
5 minute			
APGAR score	4.68 +/- 1.52	9 +/- 0	0.0001*
Expanded APGAR score	3.73 +/- 1.1	7 +/- 0	0.0001*
Combined APGAR score	8.42 +/- 2.26	16 +/- 0	0.0001*
10 minute			
APGAR score	6.1 +/- 1.55	9.6 +/- 0.49	0.0001*
Expanded APGAR score	3.78 +/- 1.08	6.87 +/- 0.48	0.0001*
Combined APGAR score	9.89 +/- 2.23	16.48 +/- 0.79	0.0001*
Blood gas parameters			
Cord pH	6.95 +/- 0.14	7.35 +/- 0.05	0.0001*
Cord base deficit (mmol/L)	15.53 +/- 4.7	4.3 +/- 1.16	0.0001*
Cord lactate (mmol/L)	10.02 +/- 3.59	3.18 +/- 1.03	0.0001*
Cord CO_2 (mm Hg)	78.08 +/- 20.03	41.4 +/- 3.8	0.0001*
Time taken to achieve pre-ductal $SpO_2 \geq 90\%$ (min)	14.94 +/- 6.47	10.06 +/- 3.88	0.006*
Time to spontaneous breathing (sec)	289.7 +/- 69.2	7.4 +/- 6.9	0.0001*
Data are represented as Mean+/-S.D. p-value <0.05 considered significant and denoted with *			

The APGAR scores and cord blood gas parameters were significantly lower in the asphyxia group when compared to controls. Time required to start of spontaneous breathing and achieving ≥90% pre-ductal saturation was significantly higher in asphyxia group.

Table 8.6: Baseline details of cases of perinatal asphyxia enrolled in study

Encephalopathy categorization (n = 19)	
HIE stage 2 (moderate encephalopathy)	15 (78.9)
HIE stage 3 (severe encephalopathy)	4 (21.1)
Cases received therapeutic hypothermia out of HIE 2 and 3 (n = 19)	
HIE 2 **HIE 3**	15/15 (100) 2/4 (50)
Evidence of clinical seizure	4 (21.1)

EEG abnormality	5 (26.3)	
Maximum resuscitation required in labour room		
	HIE stage 2 (n = 15)	**HIE stage 3 (n = 4)**
PPV	15 (100)	4 (100)
Invasive ventilation	8 (53.3)	3 (75)
Chest compression	1 (6.6)	1 (25)
IV epinephrine	1 (6.6)	0
IV fluids	0	0
Evidence of sepsis in cases - 4 (21.05)		
Meningitis	0	
Evidence of Intracranial hemorrhage in cases (n = 19)		
Overall Intra-parenchymal Other (SDH/EDH etc) Intra-ventricular	5 (26.3) 2 (10.5) 3 (15.9) 1 (5.3)	
Evidence of shock in cases in initial 72 hours	5 (26.3)	
Complications during initial management in NICU	**HIE stage 2 (n = 15)**	**HIE stage 3 (n = 4)**
SIADH	4 (26.6)	1 (25)
AKI	6 (40)	2 (50)
Raised ICP	3 (20)	2 (50)
Deranged blood sugars	1 (6.6)	0 (0)
Elevated CK-MB	15 (100)	4 (100)
Transaminitis	9 (60)	3 (75)
Data are represented as n (%), n (%) denotes the number of total subjects with their percentage in brackets		

In asphyxia cases group 15 (78.9%) and 4 (21.1%) infants were labeled as HIE-2 and HIE-3 respectively. Only 2 (50%) infants in the HIE-3 group received therapeutic hypothermia in view of persistence of hemodynamic instability in rest 2 cases. 11 (57.8%) and 2 (10.5%) of infants required invasive ventilation and chest compression respectively during initial resuscitation. 4 (21.05%) infants had evidence of early onset neonatal sepsis and 5 (26.3%) required vasopressor support for shock in asphyxia group. 5 (26.3%) neonates had evidence of intracranial hemorrhage noticed on imaging.

8.2: Primary and secondary outcome measurements:

Table 8.7: Comparison of interleukin in asphyxia and normal neonates at various time points

Parameter	Asphyxia group (n= 19)	Normal neonates (n = 33)	p-value
IL-6 (pg/ml)			
Cord	257.1 (44.5, 697.9)	59.9 (33.5, 156.7)	0.079
6 hours	116.3 (48.7, 434.2)	29.9 (15.4, 57.9)	0.001*
24 hours	170.1 (59.6, 232.1)	19.1 (11.7, 25.9)	0.0001*
72 hours	56.5 (14.6, 102.7)	5.3 (3.85, 9.5)	0.0001*
IL-1β (pg/ml)			
	17.9 (7.8, 45.6)	12.2 (0.91, 56.91)	0.126
Cord	29.8 (12.3, 65.9)	9.7 (3.99, 22.8)	0.001*
6 hours	49.8 (19.4, 132.2)	6.9 (2.55, 13.8)	0.0001*
24 hours	22.2 (5.7, 66.8)	2.09 (0.56, 5.55)	0.0001*
72 hours			
1 infant died at 24 hours of life in asphyxia group, so 72 hours analysis consists of 18 infant's data in asphyxia group. *Data is represented as Median and IQR, as M (Q1, Q3); P-value <0.05 is considered significant and denoted with *.*			

IL-6 and IL-1β values were taken at different time points post delivery in both groups. Statistically significant difference was found in IL-6 and IL-1βvalues done at 6 hours [IL-6 (p-value = 0.001, r = 0.462);IL-1β (p-value = 0.001, r = 0.471)], 24 hours [IL-6 (p-value = 0.0001, r = 0.794); IL-1β (p-value = 0.0001, r = 0.682)] and 72 hours [IL-6 (p-value = 0.0001, r = 0.702); IL-1β (p-value = 0.0001, r = 0.648)]of life between the two groups.

Table 8.8: Cytokine pattern variation with death in perinatal asphyxia group

Parameter	Death (n = 1)	Alive (n = 18)	p-value
IL-6 (pg/ml)			
0 hours	257.1	260.5 (44.5, 697.9)	1.000
6 hours	1734.4	103.9 (48.7, 244.5)	0.144
24 hours	2789.4	134.4 (59.6, 203.9)	0.100
72 hours	death	56.5 (14.6, 102.7)	-
IL-1β (pg/ml)			
0 hour	17.88	16.99 (7.8, 45.6)	1.000
6 hours	364.4	27.5 (12.3, 57.8)	0.201
24 hours	531.8	48.8 (19.4, 84.5)	0.201
72 hours	death	22.2 (5.7, 66.8)	-
Only 1 infant died, so in that group median, IQR etc. is not available. *Data is represented as Median and IQR, as M (Q1, Q3); P-value <0.05 is considered significant.*			

Out of 19 neonates with asphyxia, 1 neonate had severe asphyxia with history of meconium stained liquor with severe secondary PPHN due to meconium aspiration syndrome who died at 24 hours of life. There was a progressive increase in cytokines (IL-6) from 6 hours onwards, though not statistically significant in the cytokines level in neonate who died as compared to other neonates with asphyxia who remained alive till follow-up.

Table 8.9: Comparison of cytokine pattern with severity of asphyxia (HIE 2 Vs HIE 3)

Parameter	HIE -2 (n =15)	HIE -3 (n =4)	p-value
IL-6 (pg/ml)			
Cord	296 (65.5, 836.3)	130.2 (2.1, 477.5)	0.230
6 hours	116.3 (60, 244.5)	217.5 (0.5, 1084.3)	0.689
24 hours	170.1 (59.6, 203.9)	217.8 (64.5, 1573.2)	0.617
72 hours	44.2 (10.6, 167.1)	69 (#)	0.953
IL-1β (pg/ml)			
Cord	22.3 (9.1, 45.6)	11.7(4.1, 82.6)	0.317
6 hours	25.2 (9.4, 65.9)	46.2 (33.95, 205.8)	0.424
24 hours	47.8 (19.4, 78.9)	449.3 (188.3, 788.4)	0.134
72 hours	12.6 (4.5, 65.9)	71.9 (#)	0.110
1 neonate died at 24 hours in HIE-3 group. *Data is represented as Median and IQR, as M (Q1, Q3); P-value <0.05 is considered significant.* *# Q1, Q3 are not measurable as 1 neonate died, only 3 neonate left in that group at 72 hours of life*			

There was a progressive rise in both interleukins (IL-6 and IL-1β levels) in HIE stage 3 neonates and this progressive increase was predominant in neonate with HIE 3 not received therapeutic hypothermia eventually succumbing to illness at 24 hours of life. Though the data was not statistically significant the p-value was progressively decreasing in IL-1βfrom 24 hours onwards with a better sample effect size.

Table 8.10: Correlation of cytokines at various time points with HNNE optimality score and optimality status at discharge from NICU

	Total optimality score (HNNE)		Suboptimality status (HNNE)	
Parameter	**Correlation coefficient (ρ)**	**p-value**	**Correlation coefficient (ρ)**	**p-value**
IL-6				
cord	-0.239	0.092	0.223	0.116
6 hours	-0.330	0.018*	0.281	0.046*

24 hours	-0.786	0.0001*	0.651	0.0001*
72 hours	-0.735	0.0001*	0.561	0.0001*
IL-1β				
cord	-0.280	0.046*	0.190	0.183
6 hours	-0.380	0.006*	0.384	0.005*
24 hours	-0.602	0.0001*	0.676	0.0001*
72 hours	-0.617	0.0001*	0.649	0.0001*
1 neonate died at 24 hours of life in asphyxia group, so asphyxia group left with 18 neonates for follow-up. *Data is represented as Median and IQR, as M (Q1, Q3); P-value <0.05 is considered significant and denoted as *.*				

IL-6 values at 6 hours shows weak correlation with HNNE optimality score and suboptimality status at discharge whereas IL-6 values at 24 and 72 hours had significant strong level correlation. IL-1β showed weak correlation between 6 hours value and HNNE optimality score and suboptimality status at discharge whereas association of 24 and 72 hours IL-1β value was moderate level significant.

Table 8.11: HNNE and HINE optimal scores and global scores comparison in between asphyxia and normal neonates group

Parameter	Asphyxia group (n = 18)	Normal neonates (n = 33)	p-value
HNNE total optimality score at discharge	29.5 (26, 30.5)	34 (34, 34)	0.0001*
HINE total global score at 3-3.5 months	63.5 (58, 69)	69 (68, 70)	0.0001*
1 neonate died at 24 hours of life in asphyxia group, so asphyxia group left with 18 neonates for follow-up. *Data is represented as Median and IQR, as M (Q1, Q3); P-value <0.05 is considered significant and denoted as *.*			

Table 8.12: HINE subgroup scores comparison between asphyxia and normal neonate groups

Parameter	Asphyxia (n = 18)	Normal neonate (n = 33)	p-value
Cranial nerve function score	14 (13, 15)	15 (15, 15)	0.0001*
Posture score	14 (13, 16)	16 (16, 17)	0.0001*
Movement score	6 (5, 6)	6 (6, 6)	0.0001*
Tone score	20.5 (20, 22)	22 (22, 23)	0.0001*

Reflexes and reaction score	8 (7, 9)	9 (9, 10)	0.0001*
1 neonate died at 24 hours of life in asphyxia group, so asphyxia group left with 18 neonates for follow-up. *Data is represented as Median and IQR, as M (Q1, Q3); P-value <0.05 is considered significant and denoted as *.*			

Both the HNNE total optimality score and HINE global score were found to have statistically significant difference (p value of 0.0001 for both) with large effect sample size. Subgroup analysis of HINE was suggestive of significant difference in scores in all 5 subgroup sectors (p-value of 0.0001) with tone, movement and cranial nerve function score having medium effect sample size and effect size is larger for posture and reflexes & reactions.

Table 8.13: Correlation of IL-6 at various time points with HINE global score and optimality status at 3-3.5 months of age

	HINE global score		**HINE suboptimality status**	
Parameter	**Correlation coefficient (ρ)**	**p-value**	**Correlation coefficient (ρ)**	**p-value**
6 hours IL-6	-0.275	0.051	0.254	0.072
24 hours IL-6	-0.530	0.0001*	0.584	0.0001*
72 hours IL-6	-0.512	0.0001*	0.566	0.0001*
1 neonate died at 24 hours of life in asphyxia group, so asphyxia group left with 18 neonates for follow-up. *Data is represented as Median and IQR, as M (Q1, Q3); P-value <0.05 is considered significant and denoted as *.*				

Spearman's correlation between IL-6 values at 0, 6 hours and global score and suboptimality status assessed by HINE at 3 months of age was not statistically significant. Correlation of 24 and 72 hours IL-6 values showed moderate level association [rho = -0.530 (0.584) and -0.512 (0.566) respectively] with HINE global score and suboptimality status examined at 3 months of age.

Table 8.14: Correlation of IL-1β at various time points with HINE global score and optimality status at 3-3.5 months of age

	HINE global score		**HINE suboptimality status**	
Parameter	**Correlation coefficient (ρ)**	**p-value**	**Correlation coefficient (ρ)**	**p-value**
6 hours IL-1β	-0.227	0.110	0.343	0.014
24 hours IL-1β	-0.410	0.003*	0.558	0.0001*

72 hours IL-1β	-0.434	0.001*	0.557	0.0001*

1 neonate died at 24 hours of life in asphyxia group, so asphyxia group left with 18 neonates for follow-up.
*Data is represented as Median and IQR, as M (Q1, Q3); P-value <0.05 is considered significant and denoted as *.*

Spearman's correlation between 0 hours IL-1β value and HINE global score and suboptimality status at 3 months was not significant. 6 hours IL-1β value showed weak correlation with the suboptimality status (rho = 0.343) but association with global score was not significant. IL-1β values at 24 hours and 72 hours had moderate level correlation with global score and suboptimality status of HINE [rho = -0.41 (0.558) and -0.434 (0.557) respectively].

Table 8.15: Comparison of cytokine pattern with MRI brain abnormality in asphyxia group

Parameter	MRI abnormal (n = 10)	MRI normal (n = 8)	p-value
IL-6 (pg/ml)			
0 hours	191 (44.1, 648.1)	408.3 (84.8, 1006.5)	0.374
6 hours	90.0 (34.1, 141.5)	129.3 (82.5, 534.5)	0.214
24 hours	133.3 (66.0, 300.8)	142.7 (59.3, 201.8)	0.689
72 hours	78 (20.2, 102.7)	35.5 (10.1, 126.5)	0.424
IL-1β (pg/ml)			
Cord	20.6 (5.4, 45.1)	16.99 (9.6, 162.9)	0.424
6 hours	24.3 (21.7, 45.3)	43.8 (8.1, 78.5)	0.594
24 hours	65.8 (49.8, 366.9)	29.6 (12.3, 43.3)	0.013*
72 hours	54.7 (14.1, 71.9)	5.1 (3.3, 21.4)	0.016*
HNNE optimality score	26.5 (25, 29.5)	30.5 (29.75, 31)	0.006*
HINE global score	58.5 (54, 61)	69 (67, 69)	0.0001*
HINE subscores			
Cranial nerve function	13 (13, 15)	14.5 (14, 15)	0.063
score	13 (12, 14)	16 (16, 16.5)	0.0001*
Posture score	5 (4, 6)	6 (6, 6)	0.011*
Movement score	20 (18, 20)	22 (21.5, 23)	0.0001*
Tone score	7 (6, 8)	9 (8.5, 10)	0.006*
Reflexes and reaction score			

1 neonate died at 24 hours, so MRI of only 18 neonates in asphyxia group was done.
*Data is represented as Median and IQR, as M (Q1, Q3); P-value <0.05 is considered significant denoted with *.*

In 18 infants with evidence of moderate to severe HIE, MRI brain was done as per unit protocol. 10 neonates had features of HIE and 8 had normal MRI. 1 infant with severe HIE dies at 24 hours of life. Analysis of cytokines level between two groups suggested progressive increase of IL-1β levels from 6 hours onwards with higher values noticed at 24 and 72 hours of life which were statistically significant (p value of 0.013 and 0.016 respectively).

HNNE optimality score at discharge from NICU and HINE global score at 3 months had statistically significant difference (p value of 0.006 and 00001 respectively) between infants with abnormal MRI brain as compared to infants with normal MRI brain in asphyxia group. All components of HINE had statistically significant difference between the two groups except the cranial nerve function score. Posture and tone scores difference came out to be more statistically significant (p value of 0.0001 for each) followed by reflexes& reaction score (p-value of 0.006) and movement score (p-value of 0.011).

Table 8.16: Role of sepsis in modifying cytokine pattern in neonates with asphyxia

Parameter	Asphyxia with sepsis (n = 4)	Asphyxia without sepsis (n = 15)	p-value
IL-6 (pg/ml)			
0 hour	496.9 (170.1, 14920.7)	225.1 (44.5, 648.1)	0.368
6 hours	262.9 (75.7, 434.3)	116.3 (34.11, 244.5)	0.617
24 hours	328.9 (235.4, 820.8)	96.5 (59.6, 199.7)	0.036*
72 hours	68.9 (37.8, 301.3)	41.1 (14.6, 102.7)	0.915
IL-1β (pg/ml)			
0 hour	95.1 (23.7, 931.6)	11.7 (7.8, 45.1)	0.317
6 hours	35.3 (17.8, 249.9)	29.8 (9.4, 65.9)	0.689
24 hours	81.7 (44.3, 420.5)	47.8 (19.4, 132.2)	0.424
72 hours	40.4 (10.9, 196.7)	21.4 (4.5, 65.9)	0.339
HNNE optimality score at discharge	25.5 (24.5, 26)	30 (29, 31)	0.010*
HINE global score at 3 months	54.5 (52, 56.5)	65.5 (61, 69)	0.010*
1 neonate died at 24 hours in asphyxia without sepsis group at 24 hours. So n = 14 for 72 hours value of cytokines in that group. *Data is represented as Median and IQR, as M (Q1, Q3); P-value <0.05 is considered significant and denoted as *.*			

Among cases in asphyxia group, 4 infants developed culture proven sepsis. The difference in the mean levels of IL-6 and IL -1β though higher among babies with sepsis only IL-6 values

at 24 hours was found to be statistically significant between two groups (p = 0.036). Neonates with definite evidence of sepsis with vertical transmission in the form of sclerema neonatorum noticed at birth, had high levels of cytokines in cord blood which remained elevated even at 72 hours. On analyzing impact of sepsis on neurodevelopmental outcome, statistical difference was found in HNNE optimality score at discharge (p = 0.010) and with HINE done between 3 to 3.5 months of age (p = 0.010).

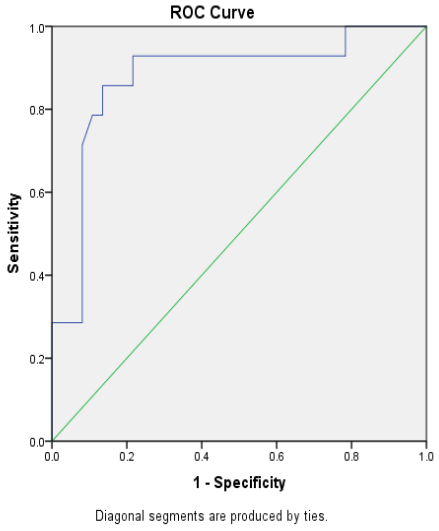
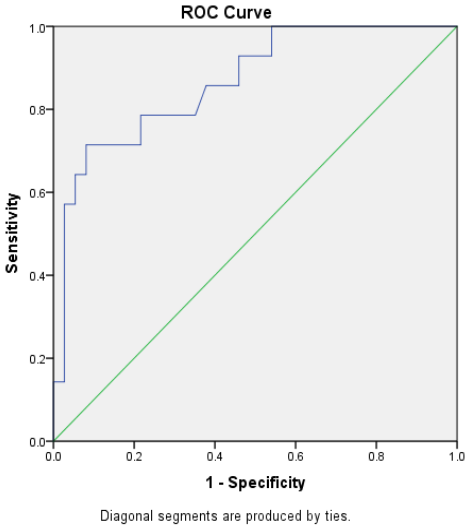
Interleukin – 6	
a. IL-6 (24 hours): Area under the curve: 0.877 95% CI: 0.75 – 0.99 With a cut-off of 48.2 pg/ml Sensitivity is 85.7% and specificity is 86.5%	**b. IL-6 (72 hours):** Area under the curve: 0.866 95% CI: 0.75 – 0.97 With a cut-off of 9.25 pg/ml Sensitivity is 85.7% and specificity is 62.2%
	

Figure 8.2: ROC curve of IL-6 levels at (a) 24 hours and (b) 72 hourswith neurodevepmental outcome at 3-3.5 months of age

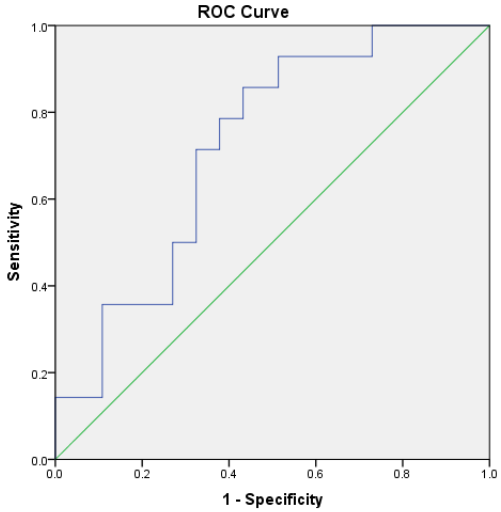
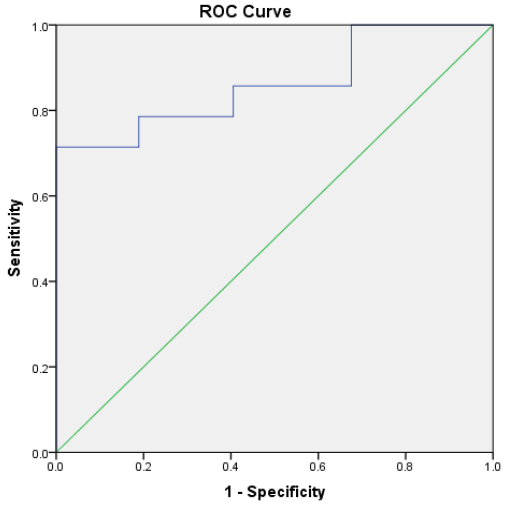
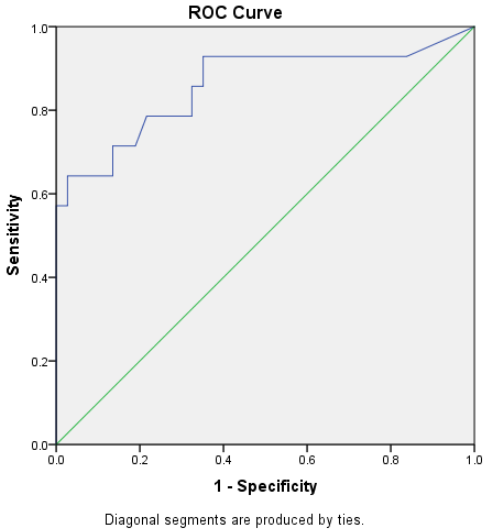
Interleukin-1β	
a. 6 hours IL-1β: Area under the curve: 0.722 95% CI: 0.57 – 0.86 With a cut-off of 12.88 pg/ml Sensitivity is 78.6% and specificity is 62.2%	**b. 24 hours IL-1β:** Area under the curve: 0.861 95% CI: 0.72 – 0.99 With a cut-off of 19.2 pg/ml Sensitivity is 78.6% and specificity is 81.1%
	
c. 72 hours IL-1β: Area under the curve: 0.860 95% CI: 0.72 – 0.99 With a cut-off of 6.29 pg/ml Sensitivity is 78.6% and specificity is 78.4%	

Figure 8.3: ROC curve of IL-1β levels at (a)6 hours, (b)24 hours and (c)72 hours with neurodevepmental outcome at 3-3.5 months of age

Table 8.17: Sensitivity and specificity of interleukins at various time points in predicting adverse outcome at 3 months (suboptimal status using HINE)

Parameter	Area under curve	95% CI	Cut-off (pg/ml)	Sensitivity	Specificity	p-value
IL-6						
24 hours	0.877	0.75-0.99	48.2	85.7	86.5	0.0001
72 hours	0.866	0.75-0.97	9.25	85.7	62.2	0.0001
IL-1β						
6 hours	0.722	0.57-0.86	12.88	78.6	62.2	0.015
24 hours	0.861	0.72-0.99	19.2	78.6	81.1	0.0001
72 hours	0.860	0.72-0.99	6.29	78.6	78.4	0.0001

Receiver operator curve (ROC curve) showed IL-6 and IL-1β levels at 24 and 72 hours are very good test to predict abnormal neurodevelopmental outcome using HINE at 3 months of age (area under the curve is >0.8). The cutoff for IL-6 at 24 hours of 48.2 pg/ml has a sensitivity and specificity of 85.7% and 86.5% respectively and a value of 31.25 pg/ml has a sensitivity and specificity of 92.9% and 78.4% to predict suboptimal score using HINE at 3 months and its value of 9.25 pg/ml at 72 hours predicts suboptimal score using HINE at 3 months with a sensitivity and specificity of 85.7 and 62.2% respectively. If the 72 hours value is decreased to 6.7 pg/ml sensitivity increases to 92.9% but specificity decreases to 54.1%, whereas upon increasing IL-6 cut-off at 72 hours to 19.45 pg/ml the sensitivity and specificity becomes 71.4% and 91.9% respectively for expecting suboptimal score with HINE at 3 months of age. IL-1β levels as early as 6 hours onwards can fairly predict the neurodevelopemntal outcome at 3 months using HINE with value of 12.88 pg/ml at 6 hours of life can predict abnormal neurodevelopmental outcome with sensitivity of 78.6% and specificity of 62.2%. 6 hours value if decreased to 9.82 then sensitivity increases to 85.7% and specificity decreases to 51.4%. For IL-1β the cutoffs at 24 hours of 19.2 pg/ml and 9.5 pg/ml has a sensitivity of 78.6% and 85.7% respectively whereas at these levels specificity will be 81.1% and 59.5% respectively. IL-1β value at 72 hours value of 6.29 pg/ml and 4.81 pg/ml predicts neurodevelopmental abnormality using HINE at 3 months with sensitivity of 78.6% and 85.7% respectively and specificity with 78.4% and 67.6% respectively.

9. DISCUSSION

9.1: STUDY BACKGROUND

In the current era of robust antenatal/perinatal follow-up with availability of better health facilities in early neonatal period has lead to decrease in mortality and morbidity associated with sepsis and other preventable causes. However prematurity and perinatal asphyxia continues to be a major burden to tackle. Perinatal asphyxia is a major causal factor for childhood morbidity and increase in disability adjusted life years (DALY)making it a significant proportion under follow-up in both neurodevelopmental clinic and early sensori-motor intervention and rehabilitation centers.

The pathogenesis of perinatal asphyxia is complex and multifactorial yet the basic mechanism behind preventable brain injury in HIE is secondary energy failure and inflammation especially in the susceptible penumbra tissue. The role of acute inflammation being central to whole pathogenesis with cytokines being the main signaling molecules under stress, the role of cytokines in neuronal damage and outcome cannot be undermined.

9.2: Primary and secondary outcomes

Clinicians and researchers are always interested in tools and ways to anticipate poor outcome and to do the risk stratification in these neonates. To classify these neonates as high risk or not various parameters are taken into account (72, 73). Cytokines being the main mechanism at cellular level for inflammation, their pattern of waxing and waning during acute phase of initial 72 hours will give a better insight into the whole process. Multiple studies were done previously trying to correlate the cytokine pattern in cord blood with neurodevelopmental outcome (74-77) but to the best of our' knowledge this is the first Indian study where the cut offs for IL-6 and IL -1βhave been defined at various time points in initial 72 hours post-delivery and this pattern was correlated with the neurodevelopmental outcome using a validated tool of HINE which can be used as early as 2 months with good sensitivity and specificity. We observed that IL-6 and IL-1βvalues from 6 hours onwards till 72 hours were significantly higher in asphyxiated neonates as compared to normal healthy neonates. IL-6 and IL-1β values at various time points though higher in severe asphyxia (HIE-3) as compared to moderate asphyxia (HIE-2), they were not statistically significant. The increase in levels of IL-6 in the peripheral blood and cord blood following perinatal asphyxia has been reported by other investigators and they found significant difference between asphyxia and normal controls (74-77). In contrast to those studies, we found that high values of IL-6 and

IL-1βcan be found in cord blood of normal neonates also who had smooth perinatal transition without any evidence of CTG abnormality or evidence of encephalopathy. However it was found that the value of IL-6 and IL-1βdecreased significantly in index study over next 6 hours making further values having significant difference between the asphyxia group and the control group. Thought here is an increase in the level of these cytokines in control group, their exact mechanism for elevation in cord blood needs to be elucidated. There is still discrepancy over whether intereukin's trans-placental transfer is possible or not with few studies refuting the same (91-93). This can be differentiated in future studies on the basis of flow cytometry analysis of cord blood as compared to maternal and fetal blood and amniotic fluid cytokine analysis. In index study there was only one death in asphyxia group and cytokine pattern difference though not significantly different from other neonates in asphyxia group it was found that there was a progressive rise in both IL-6 and IL-1β till demise which can be attributed to both primary HIE insult associated inflammation and to absence of intervention in form of therapeutic hypothermia in that neonate. In asphyxia group, IL-6levels difference was found to be statistically significant at 24 hours between neonates with evidence of sepsis as compared to neonates with asphyxia insult without any sepsis (p-value = 0.036) and IL-1β levels though not statistically significant, they were increased with associated sepsis at various time points. Cord blood levels of cytokines were very high and were in line with the severity of sepsis and features of sepsis the neonate had. For instance neonate with sclerema neonatorum had IL-6 cord value (29144 pg/ml) significantly elevated as compared to other neonates with sepsis in asphyxia group. With time the interleukin levels difference between the two groups decreased secondary to antimicrobial management. However with non-significant difference in cytokine levels in two groups majority of time suggested that it is the asphyxia component leading to significant difference in cytokine levels as compared to controls when both the groups were matched for other parameters. Both HNNE optimality score and HINE global score had statistically significant difference (p-value of 0.010 for each) between asphyxiated neonates with sepsis as compared to neonates with asphyxia without sepsis, implying the significant impact of sepsis on immediate and long term neurodevelopmental outcome. Asphyxial group cytokine pattern difference between neonates with MRI abnormality as compared to normal MRI brain found statistically significant difference in IL-1β levels at 24 and 72 hours (p-value 0.013 and 0.016 respectively).There was statistically significant difference in HNNE optimality score and HINE global score (p-value of 0.006 and 0.0001 respectively) in neonates with HIE who had asphyxia changes on MRI brain. Subcomponents score of HINE suggested significant

difference in scores between the two groups except the cranial nerve function score. IL-1βvalue difference from 24 hours onwards reinforced the significance of secondary inflammation and secondary surge of cytokines leading to local brain tissue inflammation and injury which had significant impact on overall neurodevelopmental outcome.

HINE is a simple neurodevelopmental assessment tool validated to be used between 2 months to 24 months of age (62). The HINE is easily performed and easily accessible to all clinicians with good inter-observer reliability (62).HINE optimal global score at 3-3.5 months was taken as 67 or more in index study as per the data from low risk cohort (90)though cutoff of 57 is recommended at 3 months of age by international guidelines (95, 96). HINE had sensitivity of 88% and specificity of 62% at 3 months for detection of cerebral palsy (94). Utilizing this tool we observed that IL-6 and IL -1β values had significant negative correlation with neurodevelopmental outcome assessed in the form of HNNE and HINE scores and suboptimal status at the time of discharge from NICU and at 3 months respectively. The correlation of IL-6 levels and HNNE optimal score at 6 hours was significant with a weak level negative correlation ($\rho = -0.33$) which turned into significantly good negative correlation by 24 hours ($\rho = -0.786$) and persisted with values of IL-6 at 72 hours ($\rho = -0.735$). The same pattern of correlation between IL-6 and HNNE suboptimal status was present depicting that IL-6 values starting from 6 hours onwards starts to affect the neurological status at discharge and 24 and 72 hours values has a good correlation with the neurological abnormal outcome at discharge. IL-1β values at 24 and 72 hours again had good negative correlation ($\rho = -0.602$ and -0.617 respectively) with the HNNE optimality score and had significant positive correlation with the HNNE suboptimal status ($\rho = 0.676$ and 0.649 respectively)at the time of discharge. Correlation of IL-6 at 24 and 72 hours with HINE global score found to have a significant moderate level negative correlation ($\rho = -0.53$ and -0.512 respectively). Similarly the correlation of 24 and 72 hours IL-6 levels with suboptimal status of HINE score suggested significant moderate level correlation ($\rho = 0.584$ and 0.566 respectively). The correlation of IL-1βwith HINE global score was found to be moderately significant ($\rho = -0.410$ and -0.434 respectively). IL-1β correlation with the HINE suboptimal status at 3-3.5 months found a significant moderate level correlation ($\rho = 0.558$ and 0.557 respectively).

Receiver operator curve (ROC curve) showed IL-6 and IL-1β levels at 24 and 72 hours are very good test to predict abnormal neurodevelopmental outcome using HINE at 3-3.5 months of age (area under the curve is >0.8). The cut-off for IL-6 (48.2 pg/ml) at 24 hours has a sensitivity and specificity of 85.7% and 86.5% respectively to predict suboptimal global score

using HINE at 3 months and its value of 9.25 pg/ml at 72 hours predicts suboptimal neurodevelopment status with a sensitivity and specificity of 85.7 and 62.2% respectively. Xanthou et al. found that a cut-off level for IL-6 (15.36 pg/ml)on day 1 had a sensitivity of 92.3 % and specificity of 75 % in predicting neonates at high risk of brain damage (97).

IL-1βlevels as early as 6 hours onwards can fairly predict theneurodevelopmental outcome at 3 months. Cut-off value at 6 hours of life of 12.88 pg/ml can predict abnormal neurodevelopmental outcome with sensitivity of 78.6% and specificity of 62.2%. IL-1βcutoffs at 24 hours of 19.2 pg/ml has a sensitivity and specificity of 78.6% and 81.1% and its 72 hours value of 6.29 pg/ml predicts neurodevelopmental abnormality with sensitivity and specificity 78.6% and 78.4% respectively.

9.3: Strength, limitations and implications of the study

There are a few drawbacks in the index study. In-utero cytokines level still 2nd stage of labor completion will be better suggested by amniotic fluid level of these cytokines which was not done. Being a time bound study the number of cases enrolled in asphyxia group were limited. In addition local brain tissue level injury/inflammation would have been better suggested by CSF levels of these cytokines which authors have not measured. HINE global score improves with time (90) as the infant grows and usually optimizes by 9-12 months (90), so longer neurodevelopmental follow-up is needed to correlate the pattern of cytokines with neurodevelopmental outcome in a better fashion. MRI brain when done in initial one week may miss basal ganglia and thalamus diffusion changes because of different relative coefficient of diffusion and MRS to look for local metabolite peaks would have helped. In addition MRI brain done beyond pseudo-normalization phase would have been an optimum scan delineating the exact area and volume of brain tissue injured due to insult. However, this study adds to the existing knowledge on cytokine levels in perinatal asphyxia and suggest that values of these cytokines at 24 hours and beyond will give a hint whether the inflammation process was kept under control or not thus anticipating the level of injury and risk to neurological outcome and will help in planning interventions accordingly. Correlation of outcomes of neuroprotective strategies like hypothermia, interleukin receptor blockers with cytokine levels is a potential area ofresearch though this study threw some light on progressive cytokine increase in neonates who doesn't received hypothermia and the only death occurred in the study population is of neonate who had not received therapeutic hypothermia. Secondly it also tells whether the therapeutic hypothermia was started at optimum time before inflammation sets in leading to cytokine surge. Moreover the change in

neurodevelopmental outcome secondary to Bobath and Vojta technique based early initiation of physiotherapy and occupational therapy (CIMT, SIT etc.) which relies mainly on brain's ability to reorganize(neuroplasticity) in HIE cases needs to be studied. This study also shows that IL-6 and IL-1β can be used as potential biomarkers to predict outcomes in perinatal asphyxia.

10. CONCLUSION

In our study we tried to find correlation of cytokine pattern in moderate to severe perinatal asphyxia with neurodevelopmental outcome. Though underpowered with limited asphyxia group enrolment, following conclusions were drawn from the study:

- Elevated cytokine levels (IL-6 and IL-1β) may be present in normal population in cord blood but their levels comes to normal within 6 hours if perinatal transition is smooth without any evidence of encephalopathy or peripartum depression.
- IL-6 and IL-1β levels are significantly affected by asphyxial insult during peripartum period.
- IL-6 and IL-1β values from 6 hour onwards shows significant rise in asphyxia cases which persist even at 72 hours when compared to normal control population
- It is not the initial high value of cytokine which has an impact on neurodevelopmental outcome rather the cytokines produced after secondary energy failure and inflammation in penumbra tissue leading to rise of cytokines post 6 hours of life is more important.
- Beyond 6 hours the quantification of IL-6 and IL-1β helps in risk stratification of neonates with HIE insult at birth and thus helps in predicting abnormal neurodevelopmental outcome.
- 24 hours IL-6 value of 48.2 pg/ml has a sensitivity of 85.7% and specificity of 86.5% in predicting abnormal neurodevelopmental outcome using HINE optimal global score at 3 months of age.
- 72 hours IL-6 value of 9.25 pg/ml has a sensitivity of 85.7% and specificity of 62.2% in predicting abnormal neurodevelopmental outcome using HINE optimal global score at 3 months of age.
- 6 hours IL-1β value of 12.88 pg/ml has a sensitivity of 78.6% and specificity of 62.2% in predicting abnormal neurodevelopmental outcome using HINE optimal global score at 3 months of age.
- 24 hours IL-1β value of 19.2 pg/ml has a sensitivity of 78.6% and specificity of 81.1% in predicting abnormal neurodevelopmental outcome using HINE optimal global score at 3 months of age.
- 72 hours IL-1β value of 6.29 pg/ml has a sensitivity of 78.6% and specificity of 78.4% in predicting abnormal neurodevelopmental outcome using HINE optimal global score at 3 months of age.

- .Neonates with MRI changes secondary to asphyxia has better correlation with IL-1β values 24 hours onwards with poor neurological outcome at discharge (HNNE optimality score) and at follow-up (using HINE) as compared to neonates with asphyxia without MRI changes. So IL-1β is a better marker to correlate with the MRI findings, though it needs more data to provide ample evidence regarding the same
- Sepsis has an effect on significantly altering neurological outcome at discharge assessed with HNNE andneurodevelopmental outcome assessed using HINE at 3-3.5 months.

Recommendations:

- We recommend measurement of cytokine levels (IL-6 and IL-1β) at 24 to 72 hours in HIE cases with moderate to severe encephalopathy to stratify the risk of abnormal neurodevelopmental outcome regardless of abnormal findings present on MRI or HNNE.
- We also recommend early initiation of physiotherapy and multisensory stimulation in cases with elevated cytokine levels beyond the threshold found in the study to predict abnormal outcome in HIE cases at various time points.
- We recommend to do this study on a larger cohort of asphyxia and normal population to understand the normal optimal score of HINE for both the groups in Indian population.
- More studies with interventional intent like efficiency of hypothermia or impact of Interleukin receptor antagonist and their effect on cytokine pattern needs to be done to better understand when we fail to intervene in time.

11. SUMMARY

11.1 Background

Perinatal asphyxia induced brain injury can be correlated with the start and severity of secondary energy failure and progress of inflammation leading to injury to the susceptible brain tissue in penumbra area. Cytokines having central role in inflammatory cascade, their quantification with time will help in throwing light on onset, severity and persistence of effect of initial HIE insult.

11.2 Aims & Objectives

Aim: Is there any correlation between the cytokine levels (IL-6, IL-1 beta) in newborns having perinatal asphyxia with moderate to severe HIE and their neurodevelopmental outcome at 3½ months of age?

Primary objective: To correlate cytokine pattern with neurodevelopmental assessment at 3½ months of age (using Hammersmith Infant Neurological Examination – HINE).

11.3 Materials and Methods: It was a prospective cohort study conducted in AIIMS, Jodhpur. Sequential cytokine pattern (IL-6 and IL-1β) in inborn term neonates with evidence of moderate to severe encephalopathy was compared with healthy term inborn neonates over initial 72 hours at 0, 6, 24 and 72 hours of life. The value of these cytokines were analyzed and the difference in cytokine pattern between the two groups was analyzed. If difference between the two groups was significant then plan was to correlate these cytokine pattern with the abnormal neurodevelopmental outcome at the time of discharge and at follow-up at 3-3.5 months of age using HNNE and HINE respectively.

11.4 Results

19 neonates in asphyxia group and 33 neonates in control group were enrolled. The primary outcome of correlation of cytokine pattern with abnormal neurodevelopmental outcome at 3-3.5 months of age was found to be statistically significant. IL-6 values at 24 and 72 hours had a significant moderate level correlation (ρ = 0.584 and 0.566 respectively) with abnormal outcome in the form of suboptimal score on HINE at 3 months. IL-1β correlation with the HINE sub-optimality status at 3 months found a significant moderate level correlation (ρ = 0.558 and 0.557 respectively).ROC curve showed IL-6 and IL-1β levels at 24 and 72 hours are very good test to predict abnormal neurodevelopmental outcome using HINE at 3 months

of age (area under the curve is >0.8). The cutoff for IL-6 at 24 hours of 48.2 pg/ml has a sensitivity and specificity of 85.7% and 86.5% respectively to predict suboptimal global score using HINE at 3 months and its value of 9.25 pg/ml at 72 hours predicts suboptimal neurodevelopment status with a sensitivity and specificity of 85.7 and 62.2% respectively.IL-1β levels as early as 6 hours onwards can fairly predict the neurodevelopmental outcome at 3 months with value of 12.88 pg/ml at 6 hours of life can predict abnormal neurodevelopmental outcome with sensitivity of 78.6% and specificity of 62.2%. IL-1β cutoffs at 24 hours of 19.2 pg/ml has a sensitivity and specificity of 78.6% and 81.1% and its 72 hours value of 6.29 pg/ml predicts neurodevelopmental abnormality with sensitivity and specificity 78.6% and 78.4% respectively.

11.5 Implications

Our study was able to tell that cytokines being the centre of inflammatory cascade may help in early identification of neonates at maximum risk of abnormal neurodevelopemntal outcome post HIE insult. It suggested that serum cytokines level at birth in neonates holds less significance as compared to their values at 24 hours and beyond. It also helped us in understanding the presence and severity of inflammation that happened secondary to HIE insult by looking at the pattern of cytokines between 24 to 72 hours of life, thus indicating indirectly about the timing of start and efficacy of intervention like therapeutic hypothermia in controlling the level of inflammation below a threshold and how the infant will behave neurodevelopemntally eventually helping the various rehabilitation therapies at the earliest. However, there is a need to generate more robust data by doing studies taking adequate sample size of neonates with perinatal asphyxia with HIE and looking at their long-term outcomes including mortality and neurodevelopmental disabilities.

11.6 Conclusion

The levels of IL-6 and IL -1β (at 6 hours onwards and 24 hours onwards respectively) were significantly higher in infants with perinatal asphyxia and their values at 24 and 72 hours correlated well with the adverse neurological outcome assessed using HINE at 3-3.5 months of age.

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13. ANNEXURES

13.1: PARENT INFORMATION SHEET

Name of Parent: …….

TITLE: CORRELATION OF CYTOKINE PATTERN IN MODERATE TO SEVERE PERINATAL ASPHYXIA WITH NEURODEVELOPMENTAL OUTCOME: A PROSPECTIVE COHORT STUDY

Principle Investigator: Dr. Adil Ahmed Khan, Senior Resident, Department of Neonatology.

Contact No. 9999946068/9646267950.

Co - Investigators:

Dr. Arun Kumarendu Singh, Professor and HOD, Department of Neonatology, AIIMS Jodhpur.

Dr. Neeraj Gupta, Additional Professor, Department of Pediatrics,AIIMS Jodhpur.

Dr. Pratibha Singh, Professor, Department of Obstetrics and Gynaecology, AIIMS Jodhpur

Dr. Mithu Banerjee, Additional professor and HOD, Department of Biochemistry, AIIMS Jodhpur

Place of study: Department of neonatology and Department of Obs. & Gynae, AIIMS Jodhpur.

You are invited to take part in this research study. The information in this document is meant to help you decide whether or not to take part. Please feel free to ask if you have any queries or concerns.

What is this study about?

This study is planned to know whether cytokines have a role in perinatal birth asphyxia and will try to correlate its levels with neurodevelopmental outcome.

What investigations and interventions will be done in your baby?

Your baby will undergo blood test. Blood samples will be taken during the routine sampling (additional sample required will be 1 ml). No additional pricking will be done.

What are the possible risks for your baby?

There are no significant risks associated, as this is an observational study with no additional intervention will be done or drug will be tested.

Who is paying for the research?

You will not be required to pay for the medications or lab tests; they will be borne by the research team.

Confidentiality of the information obtained from you

You have the right to confidentiality regarding the privacy of your medical information (personal details, results of physical examinations, investigations, and your medical history). By signing this document, you will be allowing the research team investigators, other study personnel, sponsors, institutional ethics committee and any person or agency required by law like the Drug Controller General of India to view your data, if required.

The results of clinical tests and therapy performed as part of this research may be included in your medical record. The information from this study, if published in scientific journals or presented at scientific meetings, will not reveal your identity.

How will your decision to not participate in the study affect you?

Your decision not to participate in this research study will not affect your medical care or your relationship with the investigator or the institution. Your doctor will still take care of your baby and you will not lose any benefits to which you are entitled.

Can you decide to stop participating in the study once you start?

The participation in this research is purely voluntary and you have the right to withdraw from this study at any time during the course of the study without giving any reasons. Though advisable that you give the investigators the reason for withdrawing, it is not mandatory

13.1 जनकसूचनापत्र

अभिवककानाम: ……

शीर्षक: मध्यम और गंभीर पेरिनाटल एस्फीक्सिया में साइटोकिन पैटर्न के साथ सहसंबंध न्यूरोडेवलपमेंटल परिणाम: भावी सहवास अध्ययन **(CORRELATION OF CYTOKINE PATTERN IN MODERATE TO SEVERE PERINATAL ASPHYXIA WITH NEURODEVELOPMENTAL OUTCOME: A PROSPECTIVE COHORT STUDY)**

सिद्धांतअन्वेषक: डॉ आदिलअहमदखान , वरिष्ठनिवासी, नियोनेटोलॉजीविभाग, एम्स, जोधपुर ।

संपर्क नंबर: 9999946068/9646267950 ।

सह - जांचकर्ता:

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डॉ प्रतिभासिंह, प्रोफेसर औरएच.ओ.डी, प्रसूतिविभागऔरस्त्रीरोगविभाग, एम्सजोधपुर

डॉ मिठू बनर्जी ,अतिरिक्तप्रोफेसरऔरएच.ओ.डी, जैवरसायनविभाग, एम्सजोधपुर

अध्ययन कि जगह: न्यूनैटॉलॉजीविभागऔरप्रसूतिविभागऔरस्त्रीरोगविभाग, एम्सजोधपुर।

आपकोइसशोधअध्ययनमेंभागलेनेकेलिएआमंत्रितकियाजाताहै। इसदस्तावेज़मेंजानकारीआपकोयहत यकरनेमेंमददकरनेकेलिएहैकि इसअध्ययनमेंभागलियाजाएयानहीं। कृपया आपअध्ययनसेसंबंधित कोईभीप्रश्नयाचिंतापूछनेकेलिएस्वतंत्रमहसूसकरें।

यहअध्ययन किस बारे में है?

इसअध्ययनसेयहपताकरनेकीयोजनाबनाईगईहैकिक्या साइटोकिन्सकीजन्मदरजन्मजातश्वासावरोध मेंभूमिकाहैऔरन्यूरोडेवलपमेंटलपरिणामकेसाथइसकेस्तरों का क्या सहसंबंध हैं ।

आपके बच्चे में क्या जांच और हस्तक्षेप किया जाएगा?

आपकाबच्चारक्तपरीक्षणसेगुजरेगा। नियमितनमूनेलेनेकेदौरानरक्तकेनमूनेलिएजाएंगे (अतिरिक्तनमूनाआवश्यकहोगा 1मिलीलीटर) । कोईअतिरिक्त चुभन नहीं होगी।

आपके बच्चे के लिए संभावित जोखिम क्या हैं?

कोईमहत्वपूर्णजोखिमनहींजुड़ेहैं,

क्योंकियहएकअवलोकनअध्ययनहै। कोईअतिरिक्तहस्तक्षेपयादवा परीक्षणनहींकियाजाएगा ।

अनुसंधान के लिए कौन भुगतान कर रहा है?

आपकोदवाओंयाप्रयोगशालापरीक्षणोंकेलिएभुगतानकरनेकीआवश्यकतानहींहोगी। यहअनुसंधानटीम केद्वाराकियाजाएगा।

आपसे प्राप्त जानकारी की गोपनीयता ?

आपको अपनी चिकित्सा जानकारी (व्यक्तिगत विवरण, शारीरिक परीक्षाओं के परिणाम, जांच और चिकित्सा इतिहास) के बारे में गोपनीयता का अधिकार है। इस दस्तावेज़ पर हस्ताक्षर करके, आप अनुसंधान दल के जांचकर्ताओं, अन्य अध्ययनकर्मियों, प्रायोजकों, नैतिक संस्थागत समिति या कानून द्वारा आवश्यक किसीभी व्यक्तिया एजेंसी, जैसे ड्रग कंट्रोलर जनरल ऑफ़ इंडिया, यदि आवश्यक हो, तोआपकाडेटादेखनेकेलिएअनुमतिदेसकते हैं।

इसशोधकेभागकेरूपमेंकिएगएनैदानिकपरीक्षणोंऔरचिकित्साकेपरिणामोंकोआपकेमेडिकलरिकॉर्डमें शामिलकियाजासकताहै। इसअध्ययनकीजानकारी, यदि वैज्ञानिक पत्रिकाओं में प्रकाशित या वैज्ञानिक बैठकों में प्रस्तुतकीजातीहै, तोआपकीपहचानकोउजागरनहींकियाजाएगा।

अध्ययन में भाग न लेने का आपका निर्णय आपको कैस प्रभावित करेगा?

इसशोधअध्ययनमें भागनहींलेने का आपकानिर्णय आपकीचिकित्सादेखभाल,अन्वेषकयासंस्थाकेसाथ आपकेसंबंधोंकोप्रभावितनहींकरेगा। आपकेडॉक्टरअभीभीआपकेबच्चेकीदेखभालकरेगेऔरआपकोईला भनहींखोएंगेजिसकेआपहकदारहैं।

क्याआप शुरू करने के बाद अध्ययन में भाग लेना बंद करने का निर्णय ले सकते हैं?

इसशोधमेंभागीदारीविशुद्धरूपसेस्वैच्छिकहैऔरआपकोअध्ययनकेदौरानकिसीभीसमय, बिनाकिसीकारणसहभागितावापसलेनेकाअधिकारहै। यह सलाहदीजातीहैकिआपजांचकर्ताओंकोसहभा गितावापसलेनेकाकारणदें,हालांकिये अनिवार्यनहींहै।

13.2: Informed Consent Form

TITLE OF THESIS/DISSERTATION: CORRELATION OF CYTOKINE PATTERN IN MODERATE TO SEVERE PERINATAL ASPHYXIA WITH NEURODEVELOPMENTAL OUTCOME: A PROSPECTIVE COHORT STUDY

Name of DM Student: **DR Adil Ahmed Khan** Tel. No. **9646267950**

Patient UHID No. : _____

I, _____ S/o or D/o _____

R/o

_____

Giving my full, free, voluntary consent for my baby to be a part of the study "**correlation of cytokine pattern in moderate to severe perinatal asphyxia with neurodevelopmental outcome: a prospective cohort study"** the procedure and nature of which has been explained to me in my own language to my full satisfaction. I confirm that I have had the opportunity to ask questions.

I understand that my participation is voluntary and I am aware of my right to opt out of the study at any time without giving any reason.

I understand that the information collected about my baby and any of my medical records may be looked at by responsible individual from AIIMS Jodhpur or from regulatory authorities. I give permission for these individuals to have access to my baby's records.

Date : _____ _____

Place : _____ Signature/Left thumb impression

This to certify that the above consent has been obtained in my presence

Date : _____ _____

Place : _____ Signature of PG Student

1. Witness 1

   _____

   Signature

   Name: _____

   Address : _____

2. Witness 2

   _____

   Signature

   Name: _____

   Address : _____

13.2: सूचित सहमति पत्र

थीसिस / शोधप्रबंध का शीर्षक: मध्यम और गंभीर पेरिनाटल एस्फीक्सिया में साइटोकिन पैटर्न के साथ सहसंबंध न्यूरोडेवलपमेंटल परिणाम: भावी सहवास अध्ययन (CORRELATION OF CYTOKINE PATTERN IN MODERATE TO SEVERE PERINATAL ASPHYXIA WITH NEURODEVELOPMENTAL OUTCOME: A PROSPECTIVE COHORT STUDY)

डी.एम.छात्र का नाम :डॉ. आदिलअहमद खान संपर्क नंबर : 9646267950

रोगी पहचान संख्या : _____

मै_____पुत्र/पुत्री_____

_____निवासी_____

_____इसशोध"मध्यमऔरगंभीरपेरिनाटलएस्फीक्सियामेंसाइटोकिनपैटर्नकेसाथस हसंबंधन्यूरोडेवलपमेंटलपरिणाम: भावीसहवासअध्ययन **(CORRELATION OF CYTOKINE PATTERN IN MODERATE TO SEVERE PERINATAL ASPHYXIA WITH NEURODEVELOPMENTAL OUTCOME: A PROSPECTIVE COHORT STUDY)"** मेभागलेनेकेलिएपूर्णसहमतिदेता/देतीहूँ|मुझेइसशोधकेबारेमेविस्तारसेसमझादियागयाहै| मैंइसबातकीपुष्टिकरता/करतीहूंकिमुझेसवालपूछनेकाअवसरदियागयाहै|शोधकीप्रकृतिऔरउद्देश्यउसके संभावितजोखिम,लाभऔरविवरणविस्तारसेमूझेसमझादिएगएहै|मैंसमझता/समझतीहूंकिमेरीभागीदारी स्वैच्छिकहै|मै□किसीभीसमय कोईभीकारणदिएबिनाइसेछोडनेकेलिएस्वतंत्रहूँ।मुझेबतादियागयाहैकिइ सकाप्रभावमेरीचिकित्सायाकानूनीअधिकारपरनहीं पगा| मुझे बता दिया गया है कि मेरा विवरण या मेरे चिकित्सकीय रिकॉर्डस को एम्स जोधपुर द्वाराउपयोगमेंलियाजाएगा| मुझसे पुछे गए सवालो के उत्तर सही व संतुष्टिपूर्वक दूँगा/दूँगी | मैंउपरोक्तशोधमेंभागलेनेकेलिएसहमतहूँ |

दिनांक: _____ _____

स्थान: _____ हस्ताक्षर / बाएंअंगूठेका निशान

यहप्रमाणितकियाजाताहैकिऊपरदियागयासहमतिपत्रमेरीउपस्थितिमेंहीलियागयाहै।

दिनांक: _____ _____

स्थान: _____ निवासी छात्र का हस्ताक्षर

1. गवाह

   _____

   हस्ताक्षर

   नाम : _____

   पता : _____

2. गवाह

   _____

   हस्ताक्षर

   नाम : _____

   पता : _____

13.3 Screening Proforma

Date: …….

Serial No.……

Name: …….

HIS No. Age:	
Inclusion Criteria:	
Term neonates ≥37weeks of gestation with one or more of the following (Tick Please)	
Gestational age (as per LMP/as per dating scan)	
Fetal heart rate (HR) tracing showing Category II or III CTG; Pathological or suspicious CTG Shoulder dystocia, Fetal macrosomia Fetal malpresentation and malposition Vacuum- or forceps-assisted vaginal delivery Meconium-stained amniotic fluid Obstructed Labour Chronic hypertension and Preeclampsia or eclampsia Twin (diamniotic diachronic twins) Polyhydramnios/oligohydramnios Fetal Growth Restriction Chorioamnionitis Symptomatic COVID-19 gravid parturient Intra-partum Bleeding Severe Maternal Anemia at the time of delivery APGAR at 1minute of life Cord blood available yes/no	
Exclusion Criteria: Outborn newborn <37 weeks gestation Antenatally detected life-threatening condition of fetus (e.g. severe hydrops, lethal chromosomal abnormality, severe congenital malformation)	
NEONATE ALLOCATED UNDER: TEST CONTROL	

13.4: Case record proforma

Title - Correlation of cytokine pattern in moderate to severe perinatal asphyxia with neurodevelopmental outcome: A Prospective Cohort Study

For any query, please contact: 9999946068/9646267950/khansaab123adil@gmail.com

	Form seria lnumber		
	Newborn enrolled	1 = YES	2 = NO
	Newborn enrolled under	1 =TEST	2= CONTROL
	Consent taken	1 = Yes	2 = No
DEMOGRAPHICDETAILS			
No.	**Item**	**Variable Name**	**Code**
1	Mother UHID		
2	Mother's name		
3	Father's name		
4	Address		
5	Telephonenumber		
6	Dateof admission		
7	Timeof admission		
8	Educationalstatus-headoffamily		1. ProfessionorHonours 2. Graduate 3. Intermediateordiploma 4. High schoolcertificate 5. Middleschoolcertificate 6. Primaryschoolcertificate 7. Illiterate
9	Occupation -head offamily		1. Legislators,SeniorOfficials&Managers 2. Professionals 3. TechniciansandAssociateProfessionals 4. Clerks 5. Skilled Workers and Shop &Market Sales Workers 6. Skilled Agricultural & FisheryWorkers 7. Craft& RelatedTradeWorkers 8. Plant& MachineOperatorsandAssemblers 9. ElementaryOccupation

			10. Unemployed
10	MonthlyFamilyincome (inrupees)		3=18,497to30,830 4=30,831to46,128 6=46,129-61,662 10=61,663-123,321 12=≥123,322
11	Socioeconomic status		1 =Upper
			2 = upper middle
			3= lower middle
			4 =upper lower
			5 =lower
MATERNALDETAILS			
12	Gravida		
13	Parity		
14	LMP		
15	EDD		
16	EDD		1 =as perLMP
			2 = asper USG T1
			3 = asper USG T2
			4 = as per NBS
17	Gestational age		
18	Methodofestimation		1 =as perLMP
			2 = asper USG T1
			3 = asper USG T2
			4 = as per NBS
19	Booked status		1 = unbooked
			2 = booked
			3 = registered
			4 =supervised
			5 =unsupervised
20	Consanguinity		1 = Yes
			2 = No

21	Maternal age (yrs)		
22	Conception		1 = Spontaneous
			2 =Ovulationinduction
			3 = ART
23	Maternal pre-pregnancyweight		
24	Maternal weightgainduringpregnancy		
25	Heightofmother		
26	BMIof mother		1 =Underweight(<18.5)
			2 = Normal(18.5 to 24.9)
			3= Overweight (25 -29.9)
			4 =Obeseclass1(30 -34.9)
			5 =Obeseclass2(35 -39.9)
			6 = Obeseclass 3(40 or more)
27	Dietary habit		1=Vegetarian
			2= Non vegetarian
29	Alcohol intake		1 = Yes
			2 = No
30	Other illicitdrugintake		1 = Yes
			2 = No
31	Nameof drug/substance		

32	Medicaldisorder	1=chronicHTN 2 = diabetesmellitus type1 3 =type2diabetes 4 = MODY 5=cardi ac disease 6=hypo thyrodi sm	yes/no

		7=autoimm une disease 8=chronic medications 9 = CKD 10=TB 11 = Epilepsy/s eizure 12=COVI Dinfection 13=Neurotic/psychotic disorder 13 =Others	
33	Chronicmedication,Ifyes specifyname		
34	Obstetricproblem	1=PIH(include PEand IE) 2 = Eclampsia 3 = GDM 4=IUGR 5 = Chorioamnionitis 6=oligohydroamnios 7=polyhydroamnios 8=malformations/syndrom es 9 = multiple pregnancy (3 or more) 10 = twin pregnancy 11= Rhnegative 12=TORCH infection 13 = Hep B/Hep C/ HIV 14 =Syphilis 15=infection/sepsisi nlastweek16 = UTI 17 = APH 18=others	Yes/no
35	IUIdiagnosis		1 = 1sttrimester
			2 = 2ndtrimester
			3 = 3rd trimester

36	Bloodgroup		1 = A
			2 = B
			3 = AB
			4 = O
37	Rh positive status		1 = Yes
			2 = No
38	ICTstatus		1= Positive
			2 = Positive
39	ICTtitre,if positive		
40	TLC/DLC	1sttrimester/date	
		2nd trimester/date	
		3rd trimester/date	
41	Hb	1sttrimester/date	
		2nd trimester/date	
		3rd trimester/date	
42	Platelets	1sttrimester/date	
		2nd trimester/date	
		3rd trimester/date	
43	TSH		
44	HbA1c		
45	Triple/Quadruplescreen		1 =Lowrisk
			2=Moderaterisk
			3= High risk
46	Diagnostictest, if yes	1 = Chorionicvillus sampling	
		2 = Amniocentesis	
		3 = Cord bloodsample	
47	Abnormalityon diagnostic testing		
48	Antenatalprogesterone		1 = Yes
			2 = No
4	Antenatalsteroids		1 = Yes

9			
			2 = No
50	Steroidcourse		1= Complete
			2=Incomplete
IntrapartumIntraamnioticInfection			
51	Fever		1 = Yes
			2 = No
52	Foulsmellingliquor/purulent discharge		1 = Yes
			2 =No
53	Fetal tachycardia		1 = Yes
			2 = No
54	MaternalTLCabnormality		1 = Yes
			2 = No
55	TLC count		
56	MaternalCRP		
57	MaternalIL-6		
58	Antibiotic taken		1 = Yes
			2 = No
60	Antibioticduration		1= <3Days
			2 = 3 to7 days
			3 = >7 days
61	Anyteratogen drugintake		1 = Yes 2 = No
Complicationsbefore/duringdelivery			
63	Antepartumhemorrhage		1 = None
			2 = Placentaprevia
			3=Abruptioplacenta
			4 =Vasaprevia
			5 =Others
65	FGR		1 =Yes
			2 = No

67	FGRtype, if present		1=Symmetric
			2=Asymmetric
68	Doppler changes	Increased S/D ratio	1 = None
			2= AEDF
			3= REDF
			4 = DVchanges
			5 = CPRabnormality
			6 =Others
69	Epiduralanalgesia		1 = Yes
			2 = No
70	Analgesia duration		
71	Total IVfluids receivedprior todelivery		
72	Inductionneeded		1 = Yes 2 = No
73	Doseofinduction	Dinoprostone gel	1 = Zero dose
		Other method	2 =1 dose
			3 =2 dose
74	Augmentation		1 = Yes
			2 = No
75	Maximumdose for augmentation(oxytocin)		
76	Duration of 1st stage ofLabour		
77	Duration of2nd stageoflabour		
78	Durationof rupture ofmembrane		1 =<12 hours
			2 =12to18hours
			3 =>18 hours
			4 = Nil
79	Modeofrupture		1 = Spontaneous
			2 = ARM
			3 = Nil
80	Fetalbaselineheartrate		1 =<100

			2 =100to160
			3 = >160
81	Beattobeatvariability		1 =<5
			2 =5to 25
			3 =>25
82	Accelerations		1 = Yes
			2 = No
83	Decelerations		1 = Yes
			2 = No
84	Typeofdeceleration		1 = Early
			2=Variable
			3 = Late
85	Type ofCTG		1=Normal
			2 =Suspicious
			3 = Pathological
86	Fetal bradycardia/tachycardia		1 = Yes
			2 = No
87	Fetalbradycardia/tachycardiaduration		1= <10 min
			2 = 10 to 30min
			3= >30 min
Deliverydetails			
88	Mode ofdelivery		1 =SVD 2=Vaccuum/ForcepsassistedVD 3= Elective LSCS 4=EmgLSCS
89	Indication		1 =TOL
			2 =Difficult/prolonged 2ndstage
			3= NPOL
			4= Shoulder dystocia
			5=Cephalopelvicdisproportion
			6=Variabledecelerations

			7=Latedecelerations
			8 =Fetaldistress
			9 =Abruption
			10=Impendingeclampsia
			11 =NotwillingforTOLAC
			12 = Abnormal lie
			13= Interlocked twins
			14 =Others
90	Presentationofbaby		1=Vertex
			2 =Breech
			3 = Face
			4=Transverse
			5 =Others
91	Meconiumstainedliquor		1 = Nil
			2=Thin
			3=Thick
92	Cordaroundtheneck		1 = No
			2 =1loop
			3 => 1loop
93	Typeofanesthesia		1 = Spinal
			2 =General
94	Opioids beforeclampingcord		1 = Yes
			2 = No
Resuscitation details			
96	NewbornUHID		
97	Date ofbirth		
98	Time of birth		
99	Gender		1=Male
			2=Female
			3 = DSD

100	Driedimmediately		1 = Yes
			2 = No
101	Initialstepsrequiredto cry		1 = Yes
			2 = No
102	Resuscitationdetails		1=Noresuscitationrequired
			2 = Initialsteps only
			3 = PPV
			4= Intubationand IPPR
			5 = Chestcompression
			6=Adrenaline
			7= IVFluids
			8=Bloodtransfusion
103	Needlethoraco/pericardio/ascetic tapdone		1 = Yes
			2 = No
104	Time of firstcry		
105	Timeofspontaneousbreathing		
106	APGAR@1min		
107	APGAR@5min		
108	APGAR@10min		
109	APGAR@20min		
110	ExpandedAPGAR@1 min		
111	ExpandedAPGAR@5 min		
1	ExpandedAPGAR@10min		

12			
113	ExpandedAPGAR@20min		
114	Time of cord clamping		1 = ECC
			2 = DCC
			3=Physiological
115	Time takentoachievepreductalspo 2of 90%		
116	Cord blood gas	Ph	
		Basedeficit	
		Lactate	
		Bicarbonate	
		pCO2	
117	Neonate temperature	at 15 min	
		at 30 min	
118	Evidenceofencephalopthy		1 = Yes
			2 = No
119	Gradeofencephalopathy		1 = Nil
			2 =Mild
			3=Moderate
			4=Severe
120	Seizure		1 = No
			2= Focal
			3 =GTCS
121	Shiftingtemperature		
122	ReceivingtemperatureinNICU		
123	Respiratorysupport@ shifting		1 = Nil
			2 = Nasalprongs
			3= CPAP

			4 = PPV
			5 = IPPV/intubated
124	Birthweight		centile(intergrowth)
125	Length		centile(intergrowth)
126	OFC		centile(intergrowth)
127	AGA/SGA/LGA		1= AGA
			2 =SGA (3rd -10th centile)
			3 =SGA (< 3rdcentile)
			4= LGA
OUTCOMEMEASURES			
128	Cytokinelevelin cord blood	IL–1b	
		IL–6	
		CRP(ifdone)	
		Procalcitonin(ifdone)	
		CBC	
129	ABG/VBGwithin1 hour(if nocord bloodgas)	pH base deficitbicar	

		b l a c t a t e Pco2	
130	Grading of encephalopathy at NICU admission (as perSarnatand Sarnat)		1 =Mild 2=Moderate 3=Severe
131	BaselineThompsonscore		
132	SeizureepisodebeforeTHstart		1 = Yes 2 = No
133	THstarttime		
134	RectaltemperatureatstartofTH		
135	Time to achievehypothermia (33.5 C)needed forTH		
136	EffectiveTH coolingstartat(hroflife)		
137	Thompson scoreat6hours		
138	Cytokine levels at2-6 hoursof life (beforetherapeutic Hypothermiastarted)	IL–1b	
		IL–6	
		CRP(ifdone)	
		Procalcitonin(ifdone)	
		CBC	
139	Baselinerespiratorysupportbef oreinitiationofTH		1=NIL
			2= CPAP
			3=NIMV
			4=IPPR
140	Respiratorysupportat6hours		1 = Nil 2=CPAP 3=NIMV 4 = Intubated
14	Shockstatus		1 =No shock

1			2 =Compensatedshock 3 =Decompensated shock
142	Ionotropic support		1 = Yes 2 = No
143	Suspicionofsepsis		1 = Yes 2 = No
144	Antibioticsupport		1 = Yes 2 = No
145	CK-MBvalue6 +/-2hours		
146	Thompson scoreat12hours		
147	Respiratorysupportat12hours		1 = Nil
			2= CPAP
			3=NIMV
			4 = Intubated
148	Hemodynamicstatus at 12 hours		1 =Noshock
			2 =Compensatedshock
			3 =Decompensated shock
149	Ionotropic support		1 = Yes
			2 = No
150	Thompson scoreat24hours		
151	Cytokinelevels at 24+/-2 hours of life	IL–1b	
		IL–6	
		CRP(ifdone)	
		Procalcitonin(ifdone)	
		CBC	
152	Thompson scoreat36hours		
153	Thompson scoreat48hours		
154	Evidence ofsepsis		1 = Yes
			2 = No
155	Antibioticstarted		1 = Yes

			2 = No
156	Totaldurationofantibiotics		1 =nil
			2 =<48hours
			3 = 48to 7 days
			4=8-14days
			5 = >14 days
157	Thompson scoreat60hours		
158	Cytokinelevels at 72 +/-2 hours of life	IL–1b	
		IL–6	
		CRP(ifdone)	
		Procalcitonin(ifdone)	
		CBC	
159	Thompson scoreat72hours		
160	TH stoppagehoursoflife		
161	EncephalopathystatusatTHstoppage		1=Noencephalopathy
			2=Mildencephalopathy
			3=Moderateencephalopathy
			4 =Severeencephalopathy
162	SeizureepisodeduringTH		1 =Noepisode
			2 =<2episode
			3 =>2episode
163	NeedofantiepilepticdrugduringTH		1 = Yes
			2 = No
164	Antiepilepticdrugused	Phenobarbitone	yes /no
		Phenytoin	yes /no
		Levipril	yes /no
		Valproate	yes / no
		Midazolam	yes / no
		Other (pls mention)	yes / no
165	Totaldurationofrespiratorysupportneeded	1= Noninvasive	

		2=Invasive	
166	Totaldurationofinotropicsupport needed		
167	Needforhydrocortisone		1 = Yes
			2 = No
168	Ifyes,howmanydoses		
169	Thompson scoreat84hours		
170	MaximumThompsonscore		
171	MinimumThompsonscoreachievedpostTH		
172	USG craniumwithin24hours		
173	USGcraniumday3		
174	EEG(ifanydone)		1 = Yes 2 = No
175	EEGdoneon(dayoflife)		
176	EEGfinding		1=Normal 2=AbN epileptiform 3 = lowvoltage
177	MRIbrain(day5to21)		1 = Yes 2 = No
178	MRIbrainfinding		
179	Grade ofMRIlesion		1=Noinjury
			2 =MildInjury
			3=ModerateInjury
			4= Severe Injury
180	Neurodevelopmentaloutcome at the time of discharge	HNNEscore	1=Normal 2= Abnormal
181	HNNEOptimalityscore(absolute score)		
182	Delayin sector on HNNEexamination		1 =Global

			2=Tone
			3 =Reflexes
			4=Movements
			5= abnormal signs
			6 = Behaviour,vision andhearing
183	Outcomeatdischarge		1= Alive
			2= Died
184	Incidence ofmortality		
185	Immediatecauseof mortality		
186	Antecedentcauseofmortality		
187	Losttofollow up		1 = Yes
			2 = No
188	Mortalitybefore 3 1/2month follow-up		1 = Yes
			2 = No
190	Globalscore onHINE(absolute score)		
191	HINEexamination	Cranial nervefunctionscore(max15)	
		Posture score (max18)	
		Movementsscore(max 6)	
		Tone score(max24)	
		Reflexesand reaction score(max15)	
		Numberofasymmtrieson HINEexamination	
		Behaviouralscore	
OtherevidenceofsepsisduringNICU(investigationchart)			
193	Temperature	1=Hypothermia 2 =Mottling 3 = CPtd(>1.5 C)	
194	Timeoftemperatureinstability		
195	BloodC/S		

196	Seizure		1 = Yes
			2 = No
197	Evidenceofmeningitis		1 = Yes
			2 = No
198	CSFcells		
199	CSFC/S		
200	CSFsugar/protein		
201	Evidenceofmeningitis/ventriculitisonUSG/MRI		1 = Yes
			2 = No
202	Evidenceofshock		
203	Evidenceofpneumonia		
204	Chestxrayfinding		
205	Typeofsepsis		1=EONS 2=LONS

13.5: HNNE Proforma

Hammersmith Neonatal Neurological Examination **Term and preterms at term age**
Ricci D et al Early Hum Devel 2008 **Page 1**

Patient Name **GA** **ID** **Date**
DOB

POSTURE	arms & legs extended or very slightly flexed	legs slightly flexed	leg well-flexed but not adducted	leg well flexed & adducted near to abdomen	abnormal posture: opisthotonus a) arms flexed, b) legs extended
ARM RECOIL	arms do not flex	arms flex slowly not always; not completely	arms flex slowly; more complete	arms flex quickly and completely	arms difficult to extend; snap back forcefully
ARM TRACTION	arms remain straight; no resistance	arms flex slightly or some resistance felt	arms flex well till shoulder lifts, then straighten	arms flex to approx. 100° & maintained as shoulder lifts	flexion of arms <100°; maintained when body lifts up
LEG RECOIL	No flexion	incomplete or variable flexion	complete but slow flexion	complete fast flexion	legs difficult to extend; snap back forcefully
LEG TRACTION	legs straight - no resistance	legs flex slightly or some resistance felt	legs flex well till bottom lifts up	knee flexes & remains flexed when bottom up	flexion stays when back & bottom up
POPLITEAL ANGLE	180°	= 150°	=110°	=90°	<90°
HEAD CONTROL (1)	no attempt to raise head	infant tries: effort better felt than seen	raises head but drops forward or back	raises head: remains vertical	
HEAD CONTROL (2)	no attempt to raise head	infant tries: effort better felt than seen	raises head but drops forward or back	raises head: remains vertical; it may wobble	head upright or extended; cannot be passively flexed
HEAD LAG	head drops & stays back	tries to lift head but it drops back	able to lift head slightly	lifts head in line with body	head in front of body
VENTRAL SUSPENSION	back curved, head & limbs hang straight	back curved, head ↓, limbs slightly flexed	back slightly curved, limbs flexed	back straight, head in line, limbs flexed	back straight, limbs above body

1	.5	2	.5	3	.5	4	.5	5	
3	0	9	6	60	9	12	0	1	25-27w
1	0	6	2	61	16	12	1	1	28-29w
2	0	4	2	65	17	8	0	2	30-31w
0	0	0	2	81	4	9	0	4	32-34w
0	0	0	0	6	3	90	1	0	Full term

3	1	9	9	44	9	23	2	0	25-27w
1	1	3	4	42	15	33	0	1	28-29w
1	0	8	3	42	10	36	0	0	30-31w
0	0	2	2	54	15	25	0	2	32-34w
0	0	5	2	22	3	67	1	0	Full term

3	0	17	5	51	10	14	0	0	25-27w
7	1	14	7	45	8	18	0	0	28-29w
7	2	15	4	51	7	14	0	0	30-31w
6	2	25	0	59	4	4	0	0	32-34w
0	0	1	0	22	8	69	0	0	Full term

3	0	14	4	18	5	52	0	4	25-27w
0	0	5	2	24	5	62	0	2	28-29w
0	0	10	2	34	2	50	0	2	30-31w
0	0	9	0	38	2	49	0	2	32-34w
0	0	3	1	4	1	91	0	0	Full term

3	1	17	6	35	6	27	1	4	25-27w
1	1	17	2	36	6	35	1	1	28-29w
2	0	21	8	38	5	25	0	1	30-31w
0	4	29	10	43	2	10	0	2	32-34w
0	0	0	1	12	12	72	0	3	Full term

3	0	22	8	46	6	14	0	0	25-27w
5	1	16	5	48	7	17	1	0	28-29w
2	0	15	10	53	5	15	0	0	30-31w
2	0	26	4	49	4	13	0	2	32-34w
0	0	5	5	19	20	51	0	0	Full term

3	0	17	4	46	9	21	0	0	25-27w
0	0	13	5	46	12	24	0	0	28-29w
3	0	14	2	48	13	20	0	0	30-31w
4	0	15	4	55	4	18	0	0	32-34w
0	0	0	6	26	12	56	0	0	Full term

3	0	3	5	57	11	21	0	0	25-27w
1	2	6	4	50	13	24	0	0	28-29w
1	0	2	2	63	11	21	0	0	30-31w
0	0	4	2	77	2	15	0	0	32-34w
0	0	0	4	29	15	52	0	0	Full term

3	3	27	13	36	3	15	0	0	25-27w
3	3	18	7	40	14	15	0	0	28-29w
7	3	16	5	46	7	16	0	0	30-31w
4	0	21	4	56	0	15	0	0	32-34w
0	0	9	4	44	12	31	0	0	Full term

0	0	21	11	38	11	15	4	0	25-27w
3	0	25	8	44	8	10	0	2	28-29w
3	0	22	8	47	5	14	1	0	30-31w
2	0	17	2	56	2	19	0	2	32-34w
0	0	4	5	47	16	28	0	0	Full term

Tone pattern items

FLEXOR TONE (compare arm and leg traction)		arm flexion < leg flexion	arm flexion = leg flexion	arm flexion > leg flexion; difference ≤1 column	arm flexion > leg flexion; difference > 1 column
FLEXOR TONE (resting posture)			arms and legs generally flexed	strong arm flexion with strong leg extension *intermittent*	strong arm flexion with strong leg extension *continuous*
LEG TONE (leg traction and popliteal angle)		leg traction > popliteal angle	leg traction = popliteal angle	leg traction < popliteal angle; difference ≤1 column	leg traction < popliteal angle; difference > 1 column
HEAD CONTROL (sitting)		neck extension < neck flexion	neck extension = Neck flexion	neck extension > neck flexion; difference ≤ 1 column	neck extension > neck flexion; difference > 1 column
NECK AND AXIAL TONE (horizontal)		ventral suspension < head lag	ventral suspension = head lag	ventral suspension > head lag; difference ≤1 column	ventral suspension > head lag; difference > 1 column

1	.5	2	.5	3	.5	4	.5	5	
0	0	45	0	27	<1	27	0	1	**25-27w**
0	0	40	<1	40	0	20	<1	0	**28-29w**
0	0	34	<1	47	<1	18	0	1	**30-31w**
0	0	38	<1	36	<1	24	<1	2	**32-34w**
0	0	25	3	53	0	18	0	<1	**Full term**

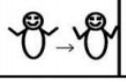
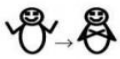
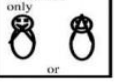
0	0	0	0	99	<1	0	0	1	**25-27w**
0	0	0	0	96	<1	3	0	1	**28-29w**
0	0	0	0	96	<1	2	0	2	**30-31w**
0	0	0	0	94	<1	2	0	4	**32-34w**
0	0	0	0	99	0	<1	0	<1	**Full term**

0	0	43	<1	34	0	21	<1	1	**25-27w**
0	0	41	0	39	<1	19	0	1	**28-29w**
0	0	38	0	36	<1	22	<1	4	**30-31w**
0	0	19	<1	50	<1	29	<1	2	**32-34w**
0	0	4	0	57	0	35	0	1	**Full term**

0	0	25	0	64	0	9	0	2	**25-27w**
0	0	17	0	70	0	13	0	0	**28-29w**
0	0	18	0	76	0	6	0	0	**30-31w**
0	0	23	0	64	0	13	0	0	**32-34w**
0	0	3	0	94	0	3	0	<1	**Full term**

0	0	20	0	39	0	35	0	6	**25-27w**
0	0	31	0	42	0	26	0	1	**28-29w**
0	0	24	0	49	0	26	0	1	**30-31w**
0	0	17	0	51	0	28	0	4	**32-34w**
0	0	24	0	58	0	18	0	<1	**Full term**

Reflex items

TENDON REFLEX	absent	felt, not seen	seen	'exaggerated'	clonus
SUCK/GAG	no gag / no suck	weak irregular suck only: no stripping	weak regular suck some stripping	strong suck: (a) irregular (b) regular good stripping	no suck but strong clenching
PALMAR GRASP	no response R L	short, weak flexion of fingers R L	strong flexion of fingers R L	strong finger flexion, shoulder ↑ R L	very strong grasp; infant can be lifted off couch R L
PLANTAR GRASP	no response R L	partial plantar flexion of toes R L	toes curve around the examiner's finger R L		
PLACING	no response R L	dorsi - flexion of ankle onl y R L	full placing response with flexion of hip, knee & placing sole on surface R L		
MORO REFLEX	no response or opening of hands only	full abduction at shoulder and extension of the arms; no adduction 	full abduction but only delayed or partial adduction 	partial abduction at shoulder and extension of arms followed by smooth adduction 	• no abduction or adduction; • only forward extension of arms from the shoulders • marked adduction only  or

1	.5	2	.5	3	.5	4	.5	5	
0	0	9	0	55	7	13	3	13	**25-27w**
0	0	12	0	50	7	22	4	5	**28-29w**
0	0	24	1	52	1	13	0	9	**30-31w**
0	0	18	0	57	0	17	4	4	**32-34w**
<1	0	21	0	78	0	<1	0	<1	**Full term**

0	0	1	0	3	3	93	0	0	**25-27w**
0	0	3	0	7	0	90	0	0	**28-29w**
0	0	0	0	6	2	92	0	0	**30-31w**
0	0	4	0	10	0	86	0	0	**32-34w**
0	0	1	0	5	0	92	0	2	**Full term**

0	0	5	0	47	7	30	1	10	**25-27w**
0	0	3	1	40	8	43	1	4	**28-29w**
0	0	1	0	51	3	35	0	10	**30-31w**
0	0	7	0	53	3	30	0	7	**32-34w**
<1	0	6	0	84	0	9	0	<1	**Full term**

0	0	4	1	95	0	0	0	0	**25-27w**
0	1	5	2	92	0	0	0	0	**28-29w**
0	0	2	1	97	0	0	0	0	**30-31w**
0	0	2	2	96	0	0	0	0	**32-34w**
<1	0	2	0	98	0	0	0	0	**Full term**

5	2	12	3	78	0	0	0	0	**25-27w**
0	2	12	6	80	0	0	0	0	**28-29w**
1	0	8	8	83	0	0	0	0	**30-31w**
0	0	4	0	96	0	0	0	0	**32-34w**
1	0	18	0	81	0	0	0	0	**Full term**

0	0	13	1	61	4	20	0	1	**25-27w**
0	0	12	1	64	6	15	1	1	**28-29w**
0	0	12	1	51	3	28	0	5	**30-31w**
0	0	23	0	46	2	27	0	2	**32-34w**
0	0	1	0	20	0	79	0	0	**Full term**

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SPONTANEOUS MOVEMENT (quantity)	no movement	sporadic and short isolated movements	frequent isolated movements	frequent generalized movements	continuous exaggerated movements
SPONTANEOUS MOVEMENT (quality)	only stretches	stretches and random abrupt movements Some smooth movements	fluent movements but mono-tonous	fluent alternating movements of arms + legs; good variability	cramped synchr-onous mouthing jerky or other abnormal movement
HEAD RAISING	no movement	infant rolls head over, chin not raised	infant raises chin, rolls head over	infant brings head and chin up	infant brings head up and keeps it up

1	.5	2	.5	3	.5	4	.5	5	
0	0	15	3	28	3	51	0	0	25-27w
0	0	17	3	26	11	43	0	0	28-29w
0	0	13	0	31	8	48	0	0	30-31w
0	0	20	0	27	0	51	0	2	32-34w
<1	0	3	0	5	0	92	0	<1	Full term

0	0	16	4	42	11	23	1	3	25-27w
0	0	22	5	35	1	23	2	2	28-29w
0	0	20	6	34	2	36	0	2	30-31w
0	0	21	0	15	0	60	0	4	32-34w
2	0	5	0	<1	0	93	0	<1	Full term

0	0	36	6	34	6	14	1	3	25-27w
1	1	35	4	34	9	14	1	1	28-29w
1	1	40	5	28	1	21	1	2	30-31w
0	0	40	0	30	4	22	2	2	32-34w
<1	0	10	0	50	0	40	0	<1	Full term

Abnormal signs

ABN. HAND OR TOE POSTURES		hands open, toes straight most of the time	intermittent fisting or thumb adduction	continuous fisting or thumb adduction; index finger flexion, thumb opposition	continuous big toe extension or flexion of all toes
TREMOR		no trem or or trem or only when crying	tremor only after Moro or occasionally when awake	frequent tremors when awake	continuous tremors
STARTLE	no startle even to sudden noise	no spontan -eous startle but reacts to sudden noise	2-3 spontaneous startles	more than 3 spontaneous startles	continuous startles

1	.5	2	.5	3	.5	4	.5	5	
0	0	57	4	37	0	2	0	0	25-27w
0	0	64	6	28	0	2	0	0	28-29w
0	0	67	1	30	1	1	0	0	30-31w
0	0	75	2	21	0	2	0	0	32-34w
0	0	85	0	12	0	3	0	<1	Full term

0	0	43	1	29	8	16	0	3	25-27w
0	0	43	0	27	9	19	2	0	28-29w
0	0	54	0	24	3	19	0	0	30-31w
0	0	62	0	30	0	4	0	4	32-34w
0	0	88	0	12	0	<1	0	<1	Full term

22	0	40	7	20	1	10	0	0	25-27w
23	1	35	7	30	2	2	0	0	28-29w
37	1	32	1	25	1	3	0	0	30-31w
50	0	35	0	9	0	6	0	0	32-34w
<1	0	94	0	6	0	<1	0	<1	Full term

EYE APPEARANCE	does not open eyes		full conjugated eye mov	transient nystagmus strabismus roving eye movemens sunsetting sign	persistent nystagmus strabismus roving eye movements dow nward deviation
AUDITORY ORIENTATION	no reaction	auditory startle; Brightens and stills; no true orientati - on	shifting of eyes, head might turn towards source	prolonged head turn to stimulus; search with eyes; smooth	turns head and eyes towards noise every time; jerky abrupt
VISUAL ORIENTATION	does not follow or focus on stimuli	stills, focuses follows briefly to the side but loses stimuli	follows horizontal -ly and vertically; no head turn	follows horizon -tally and vertically; turns head	follows in a circle
ALERTNESS	will not respond to stimuli	when awake, looks only briefly	when awake, looks at stimuli but loses them	keeps interest in stimuli	does not tire (hyper-reactive)
IRRITABILITY	quiet all the time, not irritable to any stimuli	awakes, cries some -times when handled	cries often when handled	cries always when handled	cries even when not handled
CONSOLABILITY	not crying consoling not needed	cries briefly; consol -ing not needed	cries; becomes quiet when talked to	cries; needs picking up to console	cries cannot be consoled
CRY	no cry at all	whimpe -ring cry only	cries to stimuli but normal pitch		high pitched cry; often continuous

1	.5	2	.5	3	.5	4	.5	5	
6	0	0	0	74	4	16	0	0	25-27w
2	0	0	0	80	2	15	1	0	28-29w
5	0	0	0	80	2	13	0	0	30-31w
4	0	0	0	87	2	7	0	0	32-34w
7	0	0	0	92	0	1	0	<1	Full term

5	1	28	0	57	1	8	0	0	25-27w
2	0	23	10	50	6	9	0	0	28-29w
5	1	27	7	51	1	8	0	0	30-31w
3	0	14	0	73	3	7	0	0	32-34w
<1	0	30	0	50	0	20	0	<1	Full term

6	0	7	2	25	3	26	9	22	25-27w
0	0	7	1	33	7	21	15	16	28-29w
1	0	9	0	27	5	25	10	23	30-31w
0	0	10	0	42	10	38	0	0	32-34w
<1	0	7	0	41	0	51	0	1	Full term

6	0	22	1	48	3	20	0	0	25-27w
1	0	17	4	60	3	14	1	0	28-29w
0	0	21	1	43	2	33	0	0	30-31w
0	0	7	3	54	0	36	0	0	32-34w
1	0	2	0	48	0	49	0	<1	Full term

12	1	52	0	31	0	3	0	1	25-27w
16	2	47	2	27	1	5	0	0	28-29w
27	0	47	1	22	0	2	0	1	30-31w
23	0	49	0	23	0	5	0	0	32-34w
<1	0	93	0	5	0	2	0	<1	Full term

10	0	29	0	29	3	29	0	0	25-27w
17	1	19	2	29	7	22	1	2	28-29w
27	0	18	0	28	2	22	1	2	30-31w
23	0	9	0	32	2	28	0	6	32-34w
1	0	41	0	45	0	12	0	<1	Full term

11	0	11	0	78	0	0	0	0	25-27w
16	0	5	2	77	0	0	0	0	28-29w
26	1	3	1	69	0	0	0	0	30-31w
23	0	6	2	69	0	0	0	0	32-34w
<1	0	7	0	92	0	0	0	1	Full z

Reference

Ricci D, Romeo DMM, Haataja L et al Neurological examination of preterm infants at term equivalent age. Early Human Development 2008:84;751–761

13.6: HINE Proforma

HAMMERSMITH INFANT NEUROLOGICAL EXAMINATION (v 08.02.19)

Name **Date of birth**

Gestational age **Date of examination**

Chronological age / Corrected age **Head circumference**

SUMMARY OF EXAMINATION
Global score (max 78)
Number of asymmetries
Behavioural score (not part of the optimality score)

Cranial nerve function	score	(max 15)
Posture	score	(max 18)
Movements	score	(max 6)
Tone	score	(max 24)
Reflexes and reactions	score	(max 15)

COMMENTS

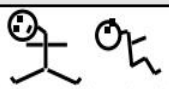
(Throughout the exam, if a response is not optimal but not poor enough to score 1, give a score of 2)

NEUROLOGICAL EXAMINATION

ASSESSMENT OF CRANIAL NERVE FUNCTION

	score 3	2	score 1	score 0	score	Asymmetry / Comments
Facial appearance (at rest and when crying or stimulated)	Smiles or reacts to stimuli by closing eyes and grimacing		Closes eyes but not tightly, poor facial expression	Expressionless, does not react to stimuli		
Eye movements	Normal conjugate eye movements		**Intermittent** Deviation of eyes or abnormal movements	**Continuous** Deviation of eyes or abnormal movements		
Visual response Test ability to follow a black/white target	Follows the target in a complete arc		Follows target in an incomplete or asymmetrical arc	Does not follow the target		
Auditory response Test the response to a rattle	Reacts to stimuli from both sides		Doubtful reaction to stimuli or asymmetry of response	No response		
Sucking/swallowing Watch infant suck on breast or bottle. If older, ask about feeding, assoc. cough, excessive dribbling	Good suck and swallowing		Poor suck and/or swallow	No sucking reflex, no swallowing		

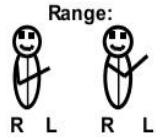
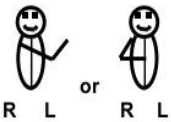
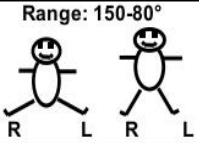
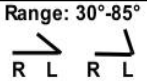
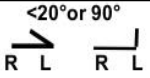
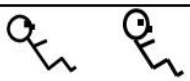
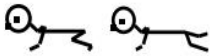
ASSESSMENT OF POSTURE (note any asymmetries)

	score 3	score 2	score 1	score 0	sc	Asymmetry / comments
Head in sitting	 Straight; in midline		 Slightly to side *or* backward *or* forward	 Markedly to side *or* backward *or* forward		
Trunk in sitting	 Straight		 Slightly curved or bent to side	 Very rounded rocketing back bent sideway		
Arms at rest	In a neutral position, central straight or slightly bent		**Slight** internal rotation *or* external rotation **Intermittent** dystonic posture	**Marked** internal rotation *or* external rotation or dystonic posture hemiplegic posture		
Hands	Hands open		**Intermittent** adducted thumb *or* fisting	**Persistent** adducted thumb *or* fisting		
Legs in sitting	Able to sit with a straight back and legs straight or slightly bent (long sitting) 		Sit with straight back but knees bent at 15-20 ° 	Unable to sit straight unless knees markedly bent (no long sitting) 		
in supine and in standing	Legs in neutral position straight *or* slightly bent	**Slight** internal rotation or external rotation	Internal rotation *or* external rotation at the hips	**Marked** internal rotation *or* external rotation *or* fixed extension or flexion or contractures at hips and knees		
Feet in supine and in standing	Central in neutral position Toes straight midway between flexion and extension		**Slight** internal rotation *or* external rotation **Intermittent** Tendency to stand on tiptoes or toes up or curling under	**Marked** internal rotation *or* external rotation at the ankle **Persistent** Tendency to stand on tiptoes *or* toes up or curling under		

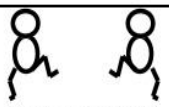
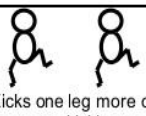
ASSESSMENT OF MOVEMENTS

	Score 3	Score 2	Score 1	Score 0	score	Asymmetry / comments
Quantity Watch infant lying in supine	Normal		Excessive or sluggish	Minimal or none		
Quality Observe infant's spontaneous voluntary motor activity during the course of the assessment	Free, alternating, and smooth		Jerky Slight tremor	• Cramped & synchronous • Extensor spasms • Athetoid • Ataxic • Very tremulous • Myoclonic spasm • Dystonic movement		

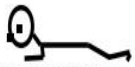
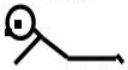
ASSESSMENT OF TONE

	Score 3	Score 2	Score 1	Score 0	sc	Asym/Co
Scarf sign Take the infant's hand and pull the arm across the chest until there is resistance. Note the position of the elbow in relation to the midline.	**Range:**  R L R L		 R L	 or R L R L		
Passive shoulder elevation Lift arm up alongside infant's head. Note resistance at shoulder and elbow.	Resistance overcomeable  R L	Resistance difficult to overcome R L	No resistance  R L	Resistance, not overcomeable  R L		
Pronation/supination Steady the upper arm while pronating and supinating forearm, note resistance	Full pronation and supination, no resistance		Resistance to full pronation / supination overcomeable	Full pronation and supination not possible, marked resistance		
Hip adductors With both the infant's legs extended, abduct them as far as possible. The angle formed by the legs is noted.	**Range: 150-80°**  R L R L	**150-160°**  R L	**>170°**  R L	**<80°**  R L		
Popliteal angle Keeping the infant's bottom on the bed, flex both hips onto the abdomen, then extend the knees until there is resistance. Note the angle between upper and lower leg.	**Range: 150°-100°**  R L R L	**150-160°**  R L	**~90° or > 170°**  R L R L	**<80°**  R L		
Ankle dorsiflexion With knee extended, dorsiflex the ankle. Note the angle between foot and leg.	**Range: 30°-85°**  R L R L	**20-30°**  R L	**<20°or 90°**  R L R L	**> 90°**  R L		
Pull to sit Pull infant to sit by the wrists. (support head if necessary)						
Ventral suspension Hold infant horizontally around trunk in ventral suspension; note position of back, limbs and head.						

REFLEXES AND REACTIONS

	Score 3	Score 2	Score 1	Score 0	sc	Asym / Co
Arm protection Pull the infant by one arm from the supine position (steady the contralateral hip) and note the reaction of arm on opposite side.	 Arm & hand extend R L		 Arm semi-flexed R L	 Arm fully flexed R L		
Vertical suspension hold infant under axilla making sure legs do not touch any surface – you may "tickle" feet to stimulate kicking.	 Kicks symmetrically		 Kicks one leg more or poor kicking	 No kicking even if stimulated or scissoring		
Lateral tilting (describe side up). Hold infant up vertically near to hips and tilt sideways towards the horizontal. Note response of trunk, spine, limbs and head.	 R L	 L R	 R L	 R L		
Forward parachute Hold infant up vertically and quickly tilt forwards. Note reaction /symmetry of arm responses,	 (after 6 months)		 (after 6 months)			
Tendon Reflexes Have child relaxed, sitting or lying – use small hammer	Easily elicitable biceps knee ankle	Mildly brisk bicep knee ankle	Brisk biceps knee ankle	Clonus or absent biceps knee ankle		

SECTION 2 MOTOR MILESTONES (not scored; note asymmetries)

Head control	Unable to maintain head upright normal to 3m	Wobbles normal up to 4m	Maintained upright all the time normal from 5m			**Please note age at which maximum skill is achieved**
Sitting	Cannot sit	With support at hips  normal at 4m	Props  normal at 6m	Stable sit  normal at 7-8m	Pivots (rotates)  normal at 9m	Observed: Reported (age):
Voluntary grasp – note side	No grasp	Uses whole hand	Index finger and thumb but immature grasp	Pincer grasp		Observed: Reported (age):
Ability to kick in supine	No kicking	Kicks horizontally but legs do not lift	Upward (vertically)  normal at 3m	Touches leg  normal at 4-5m	Touches toes  normal at 5-6m	Observed: Reported (age):
Rolling - note through which side(s)	No rolling	Rolling to side normal at 4m	Prone to supine normal at 6 m	Supine to prone normal at 6 m		Observed: Reported (age):
Crawling - note if bottom shuffling	Does not lift head	On elbows  normal at 3m	On outstretched hands  normal at 4m	Crawling flat on abdomen  normal at 8m	Crawling on hands and knees  normal at 10m	Observed: Reported (age):
Standing	Does not support weight	Supports weight normal at 4m	Stands with support normal at 7m	Stands unaided normal at 12m		Observed: Reported (age):
Walking		Bouncing normal at 6m	Cruising (walks holding on) normal at 12m	Walking independently normal by 15m		Observed: Reported (age):

SECTION 3 BEHAVIOUR (not scored)

	1	2	3	4	5	6	Comment
Conscious state	Unrousable	Drowsy	Sleep but wakes easily	Awake but no interest	Loses interest	Maintains interest	
Emotional state	Irritable, not consolable	Irritable, carer can console	Irritable when approached	Neither happy or unhappy	Happy and smiling		
Social orientation	Avoiding, withdrawn	Hesitant	Accepts approach	Friendly			

This is the official form for use with the Hammersmith Infant Neurological Examination.
Its content and scoring system are not to be changed. ***Main reference*** *Haataja L et al J Peds 1999;135:153-61*
For enquiries about the examination*, please contact Prof Frances Cowan f.cowan@imperial.ac.uk,*
Prof Leena Haataja leena.haataja@hus.fi or Prof Eugenio Mercuri eugeniomercuri@unicatt.it

13.7: Items required for HNNE and HINE examination

1. Visual Target- Red wool/Black and white target

2. Rattle/Bell

3. Neonatal Hammer

4. Goniometer

5. Torch

6. Non stretchable measuring tape

(For visual fixation and following black and white circle pattern is used; Wool avoided in neonates during HNNE examination in view of fine threads creating diffraction and dispersion and thus creating confusion in neonates sensory pathway leading to aberrant motor response to stimuli)

13.8: Definitions used in study

1. **Term neonate-** Those born at ≥37 weeks of gestation.
2. **Early term-** Those born between 37 weeks and 0 day to 38 weeks and 6 days of gestation.
3. **Full term-** Those born at 39 weeks and 0 day to 40 weeks and 6 days of gestation.
4. **Late term-** Those born at 41 weeks and 0 day to 41 weeks and 6 days of gestation.
5. **Healthy term infants** –37+0 weeks and 0 day till 41+6 weeks. These were those neonates who were normal and shifted with mother side and stays with her till discharge at 72 hours or more without any complication during admission duration satisfying the following criteria:

   A). No major congenital or chromosomal anomalies

   B). No evidence of perinatal asphyxia (cried immediately after birth or post initial steps and not requiring any resuscitation with Apgar at 1 minute of 7 or more).

   C). No evidence of encephalopathy secondary to any disorder/ insult.

   D). Non vacuum assisted delivery/ difficult extraction.

   E). No delayed second stage of labour.

   F). No evidence of pathological decelerations.

   G). No evidence of sepsis/intrauterine infection in mother.

   H). No evidence of EONS in neonate.

   I). No signs of serious birth injury/cephalhematoma.

   J). No evidence of pathological jaundice.
6. **Perinatal asphyxia**–NNPD network definition is used for labeling neonates as no asphyxia, moderate asphyxia or severe asphyxia:

NNPD Network	**Moderate PA:** Slow/gasping breathing or an APGAR of 4 to 6 at 1 minute of life
	Severe PA: No breathing or APGAR of 0-3 at 1 minute of life

7. Peripartum depression: Pertains to the condition of the infant on physical examination in the immediate postnatal period, i.e., in the first hour after birth. The clinical features of infants with this condition may include depressed mental status, muscle hypotonia, and/or disturbances in spontaneous respiration and cardiovascular function. This term makes no association with the prenatal or later postnatal (beyond the first hour) condition, physical examination, laboratory tests, imaging studies or EEG. (If condition persisting beyond first hour, the state of infant will be labeled as neonatal encephalopathy based on modified Sarnat and Sarnat staging into mild, moderate or severe encephalopathy.

8. Hypoxic ischemic encephalopathy: Neonates who have clinical evidence of encephalopathy in the form of altered level of consciousness and usually other signs of brainstem and/or motor dysfunction persisting beyond first hour of life secondary to delayed cry at birth requiring resuscitation and with objective data to support a hypoxic-ischemic mechanism as the underlying cause for the encephalopathy. To give therapeutic hypothermia in HIE cases, we follow NICHD criteria in our NICU. To label encephalopathy secondary to hypoxic-ishemic insult into mild, moderate or severe, we use Modified Sarnat and Sarnat staging.

9. End organ injury: For the purpose of this study, end organ injury secondary to perinatal asphyxia will be defined as evidence of either of elevated CK-MB (Senthil K. et al 2017) or elevated creatinine/decrease urine output or evidence of cerebral edema (leading to seizures, SIADH, Cushing's reflex triadetc) or evidence of feed intolerance/altered aspirates/abdominal distension/absent bowel sounds.

10. Resuscitation: For the purpose of this study, resuscitation is defined as any effort to initiate or sustain breathing/cryingand heart rate >100 /min, if required beyond initial steps

11. Initial stepsduring resuscitation: Defined as per NRP® 8th edition as sequence: warm, dry, stimulate, position airway, suction (if needed).

12. Neonatal sepsis

A. Early onset neonatal sepsis (EONS) – onset before 72 hours of life.

B. Late onset neonatal sepsis (LONS) – onset after 72 hours of life.

13. Risk factor for early onset neonatal sepsis*:

A.Spontaneous prematurity

B. Foul smelling liquor

C. Rupture of membranes >24 hours

D. Single unclean or >3 sterile vaginal examinations during labour

E. Prolonged labour (duration of 1st and 2nd stage of labour of 24 hrs or more)

F. Perinatal asphyxia (Apgarscore of <4 at 1 min of life)

(*Presence of foul smelling liquor or three of the above mentioned risk factors if present, warrants initiation of antibiotics)

14. Chorioamnionitis (Intra-amniotic infection): Defined as fever (>38 degree Celsius) plus 2 of:

A.Maternal tachycardia

B. Fetal tachycardia

C. Uterine tenderness

D. Foul smelling vaginal discharge during labour

E. Maternal WBCs≥15000/mm3 in absence of corticosteroids

15. HNNE: Hammersmith Neonatal Neurological Examination (HNNE) is an internationallyvalidated objective method, used for the neurological assessment of term and preterm infants. Itis a simple and scorable method designed for evaluating neurological examination among preterm and term neonates. It encompasses 34 items assessing 6 categories qualitatively and quantitatively(tone, tone pattern, reflexes, spontaneousmovements, abnormal signs and behavioural items).

16. Optimality score: Optimality score is based on the frequency distribution of the scores in thenormal population, defining as optimal all the scores found in atleast 90% of a cohort of normalhealthy population.

17. Optimality score in HNNE: Optimality score in HNNE is a method to assess neonatal neurological status quantitatively based on neonatal neuro-behavioural examination which can be usedto compare the neurological differences between preterm/at risk and term neonates. Optimality score forindividual item of HNNE proforma is calculated based on frequency distribution of the rawscores in normal population and total OS is calculated by adding the optimality score of all 34items. For a given item, most frequently observed findings or findings seen in ≥90% ofpopulation or falling more than 10th centile is defined as optimal. Any item seen in less than 5%population or falling less than 5th centile is regarded as suboptimal. Items seen between 5-10%of population are classed as borderline.

18. **Hammersmith Infant neurological examination (HINE):** It is based on the same principle as HNNE examination (based on Dubowitz et l, 1981, neonatal neurological examination of at risk infants who are prone for neurological abnormalities). Hammersmith Infant Neurological Examination (HINE) is an internationally validated objective method, used for the neurological assessment of term and preterm infants and their neurological impairment. It is a simple and scorable method for assessing infants between 2 to 24 months of age including items under 5 categories(cranial nerve function, posture, tone, movements and reflexes).

19. **Optimality score in HINE:** The optimality score is obtained by calculating the distribution of frequency of the scores in the normal population, defining as optimal all the scores found to be inatleast 90% of the cohort. The overall score ranges from minimum 0 to maximum 78.

13.9: APPENDICES

Appendix 13.9.1: CTG classification (NICE 2020)

Description	Feature		
	Baseline (beats/ minute)	**Baseline variability (beats/ minute)**	**Decelerations**
Reassuring	110 to 160	5 to 25	None or early Variable decelerations with no concerning characteristics* for less than 90 minutes
Nonreassuring	100 to 109† OR 161 to 180	Less than 5 for 30 to 50 minutes OR More than 25 for 15 to 25 minutes	Variable decelerations with no concerning characteristics* for 90 minutes or more OR Variable decelerations with any concerning characteristics* in up to 50% of contractions for 30 minutes or more OR Variable decelerations with any concerning characteristics* in over 50% of contractions for less than 30 minutes OR Late decelerations in over 50% of contractions for less than 30 minutes, with no maternal or fetal clinical risk factors such as vaginal bleeding or significant meconium
Abnormal	Below 100 OR Above 180	Less than 5 for more than 50 minutes OR More than 25 for more than 25 minutes OR Sinusoidal	Variable decelerations with any concerning characteristics* in over 50% of contractions for 30 minutes (or less if any maternal or fetal clinical risk factors [see above]) OR Late decelerations for 30 minutes (or less if any maternal or fetal clinical risk factors) OR Acute bradycardia, or a single prolonged deceleration lasting 3 minutes or more

Abbreviation: CTG, cardiotocography.

* Regard the following as concerning characteristics of variable decelerations: lasting more than 60 seconds; reduced baseline variability within the deceleration; failure to return to baseline; biphasic (W) shape; no shouldering.

† Although a baseline fetal heart rate between 100 and 109 beats/minute is a non-reassuring feature, continue usual care if there is normal baseline variability and no variable or late decelerations

Appendix 13.9.2: Management based on CTG traces:

Category	Definition	Management
Normal	All features are reassuring	• Continue CTG (unless it was started because of concerns arising from intermittent auscultation and there are no ongoing risk factors; see recommendation 1.10.8) and usual care • Talk to the woman and her birth companion(s) about what is happening
Suspicious	1 non-reassuring feature AND 2 reassuring features	• Correct any underlying causes, such as hypotension or uterine hyperstimulation • Perform a full set of maternal observations • Start 1 or more conservative measures* • Inform an obstetrician or a senior midwife • Document a plan for reviewing the whole clinical picture and the CTG findings • Talk to the woman and her birth companion(s) about what is happening and take her preferences into account
Pathological	1 abnormal feature OR 2 non-reassuring features	• Obtain a review by an obstetrician and a senior midwife • Exclude acute events (for example, cord prolapse, suspected placental abruption or suspected uterine rupture) • Correct any underlying causes, such as hypotension or uterine hyperstimulation • Start 1 or more conservative measures* • Talk to the woman and her birth companion(s) about what is happening and take her preferences into account • If the cardiotocograph trace is still pathological after implementing conservative measures: – obtain a further review by an obstetrician and a senior midwife – offer digital fetal scalp stimulation (see recommendation 1.10.38) and document the outcome • If the cardiotocograph trace is still pathological after fetal scalp stimulation: – consider fetal blood sampling – consider expediting the birth – take the woman's preferences into account
Need for urgent intervention	Acute bradycardia, OR a single prolonged deceleration for	• Urgently seek obstetric help If there has been an acute event (for example, cord prolapse, suspected placental abruption or suspected uterine rupture), expedite the birth • Correct any underlying causes, such as hypotension or

	3 minutes or more	uterine hyperstimulation • Start 1 or more conservative measures* • Make preparations for an urgent birth • Talk to the woman and her birth companion(s) about what is happening and take her preferences into account • Expedite the birth if the acute bradycardia persists for 9 minutes • If the fetal heart rate recovers at any time up to 9 minutes, reassess any decision to expedite the birth, in discussion with the woman
Abbreviation: CTG, cardiotocography. * If there are any concerns about the baby's wellbeing, be aware of the possible underlying causes and start one or more of the following conservative measures based on an assessment of the most likely cause(s): encourage the woman to mobilise or adopt an alternative position (and to avoid being supine); offer intravenous fluids if the woman is hypotensive; reduce contraction frequency by reducing or stopping oxytocin if it is being used and/or offering a tocolytic drug (a suggested regimen is subcutaneous terbutaline 0.25 mg).		

Appendix 13.9.3: Criteria to label perinatal asphyxia case as moderate to severe HIE

Hypoxic ischemic encephalopathy: Neonates who have clinical evidence of encephalopathy in the form of altered level of consciousness and usually other signs of brainstem and/or motor dysfunction persisting beyond first hour of life secondary to delayed cry at birth requiring resuscitation and with objective data to support a hypoxic-ischemic mechanism as the underlying cause for the encephalopathy. To give therapeutic hypothermia in HIE cases, we follow NICHD criteria in our NICU.

To label encephalopathy secondary to hypoxic-ishemic insult into mild, moderate or severe, we use Modified Sarnat and Sarnat staging.

Inclusion Criteria: Inborn term newborns (≥37 week period of gestation) with perinatal asphyxia (as per NNPD) with evidence of moderate to severe HIE as per the following criteria:

Criteria for Neonates with Eligibility for Inclusion	
Biochemical Criteria	Blood gas within 1st hour of life o (Cord, ABG, CBG, VBG) pH ≤ 7 **OR** base deficit ≥ 16
	Additional Criteria o If NO blood gas result available o **OR** o 1st hour gas pH is between 7.01-7.15 o **OR** o Base deficit 10-16 o **AND both of the following** o 10 min Apgar ≤ 5 **OR** assisted ventilation at birth continued for ≥ 10 minutes ▪ Not limited to intubation and mechanical ventilation, may include CPAP o An acute perinatal event: ▪ Late and/or variable decelerations, ▪ Cord prolapse/rupture ▪ Uterine rupture ▪ Maternal trauma, hemorrhage, cardiorespiratory arrest ▪ Shoulder dystocia ▪ Nuchal cord ▪ Other event leading to occurrence of hypoxia

Once these criteria were met, all infants will undergo a standardized neurologic examination. Infants are candidates for the study when encephalopathy or seizures were present.

Encephalopathy will be defined as the presence of one or more signs in at least three of the following six categories: level of consciousness, spontaneous activity, posture, tone, primitive reflexes (suck or Moro), and autonomic nervous system (pupils, heart rate, or respiration).

The number of moderate or severe signs will determine the extent of encephalopathy as defined by modified Sarnat staging; if signs of moderate and severe encephalopathy are equally distributed, then the labeling will be based upon level of consciousness.

Criteria for defining moderate and severe encephalopathy		
Category	**Moderate encephalopathy**	**Severe encephalopathy**
Level of consciousness	Lethargic	Stupor or coma
Spontaneous activity	Decreased activity	No activity
Posture	Distal flexion, complete extension	Decerebrate
Tone	Hypotonia (focal or general)	Flaccid
Primitive reflexes Suck Moro	 Weak Incomplete	 Absent Absent
Autonomic system Pupils Heart rate Respiration	 Constricted Bradycardia Periodic breathing	 Deviated, dilated or non reactive to light Variable Apnea

Appendix 13.9.4: Sarnat and Sarnat Staging to label level of encephalopathy

Stage	Stage 1 (Mild)	Stage 2 (Moderate)	Stage 3 (Severe)
Level of consciousness	Hyperalert; irritable	Lethargic or obtunded	Stuporous, comatose
Neuromuscular control:	Uninhibited, overreactive	Diminished spontaneous movement	Diminished or absent spontaneous movement
Muscle tone	Normal	Mild hypotonia	Flaccid
Posture	Mild distal flexion	Strong distal flexion	Intermittent decerebration
Stretch reflexes	Overactive	Overactive, disinhibited	Decreased or absent
Segmental myoclonus	Present or absent	Present	Absent
Complex reflexes:	Normal	Suppressed	Absent
Suck	Weak	Weak or absent	Absent
Moro	Strong, low threshold	Weak, incomplete high threshold	Absent
Oculovestibular	Normal	Overactive	Weak or absent
Tonic neck	Slight	Strong	Absent
Autonomic function:	Generalized sympathetic	Generalized parasympathetic	Both systems depressed
Pupils	Mydriasis	Miosis	Midposition, often unequal; poor light reflex
Respirations	Spontaneous	Spontaneous; occasional apnea	Periodic; apnea
Heart rate	Tachycardia	Bradycardia	Variable

Bronchial and salivary secretions	Sparse	Profuse	Variable
Gastrointestinal motility	Normal or decreased	Increased diarrhea	Variable
Seizures	None	Common focal or multifocal (6 to 24 hours of age)	Uncommon (excluding decerebration)
Electroencephalographic findings	Normal (awake)	Early: generalized low-voltage, slowing (continuous delta and theta)	Early: periodic pattern with isopotential phases
		Later: periodic pattern (awake); seizures focal or multifocal; 1.0 to 1.5 Hz spike and wave	Later: totally isopotential
Duration of symptoms	<24 hours	2 to 14 days	Hours to weeks
Outcome	About 100% normal	80% normal; abnormal if symptoms more than 5 to 7 days	About 50% die; remainder with severe sequelae
* The stages in this table are a continuum reflecting the spectrum of clinical states of infants over 36 weeks' gestational age.			
Source: From Sarnat H. B., Sarnat M. S. Neonatal encephalopathy following fetal distress: A clinical and electroencephalographics study. *Arch Neurol* 1976;33:696.			

Appendix 13.9.5: Thompson score

SIGN	SCORE			
	0	1	2	3
Tone	Normal	Hyper	Hypo	Flaccid
Level of Consciousness	Normal	Hyperalert, Stare	Lethargic	Comatouse
Fits	None	Infrequent, <3 per hour	Frequent >2 per hour	
Posture	Normal	Fisting, Cycling	Strong distal flexion	Decerbrate
Moro Reflex	Normal	Partial	Absent	
Grasping	Normal	Poor	Absent	
Sucking	Normal	Poor	Absent ± bites	
Breathing	Normal	Hyperventilation	Brief apnea	IPPV (apnea)
Fontanelle	Normal	Full, not tense	Tense	
Date and time TOTAL:				

Appendix 13.9.6:MRI injury scoring in neonatal HIE cases: (Trivedi et al 2017)

<table>
<tr><th>REGION</th><th>SCORE</th></tr>
<tr><td>1. Subcortical region
a) caudate nucleus
b) globus pallidus/putamen
c) thalamus
d) posterior limb of internal capsule

2. White matter

3. Cortex

4. Cerebellum

5. Brainstem</td><td>0 = no signal abnormality
1 = signal abnormality in <25% of the region
2 = signal abnormality in 25-50% of the region
3 = widespread injury involving >50% of the region

On T1 and T2 weighted image the PLIC is graded from:
0 = well myelinated (>50%)
1 = partially myelinated (25-50%)
2 =minimal myelination (<25%)
3 = absent myelination

On diffusion weighted imaging, the PLIC is graded on the area of diffusion restriction from no restriction (0) to extensive restricted diffusion (3)

Brainstem scoring is done from (0) to maximum (2)</td></tr>
<tr><td colspan="2">Regional subscore: summation of independent T1 + T2 + DWI images for the region</td></tr>
<tr><td colspan="2">MRI injury grade: summation of the 5 regional subscores</td></tr>
<tr><td>0 = No injury</td><td>12-32 = Moderate injury</td></tr>
<tr><td>1-11 = Mild injury</td><td>33-138 = Severe injury</td></tr>
</table>

Appendix 13.9.7: Volpe's Intraventricular hemorrhage (IVH) grading system

Grading system	Severity of IVH/GMH	Description/ findings
Volpe	**I**	GMH with no or minimal IVH (<10% ventricular volume)
	II	IVH occupying 10-50% of ventricular area on parasagittal view
	III	IVH occupying >50% of the ventricular area on parasagittal view, usually distends lateral ventricle (at the time of IVH diagnosis)
	IPE	Intraparenchymal/ periventricular echodensities (location and extent)

Appendix 13.9.8: CK-MB nomogram used to label level of myocardial injury in current study (Senthil K. et al 2017)

CK-MB correlation (Senthil K. et al 2017)	
Normal values (u/L):	
Weight wise	**Delivery mode**
<2.5 kg = 30.2 **2.6 -3 kg** = 25.38 **>3 kg** = 35	**LSCS** = 22.57 **Natural** = 27.6 **Forceps/vacuum** = 35
For the purpose of this study any CK-MB value >100 will be considered as elevated; value between 50-100 will be considered as borderline and <50 will be labelled as normal	

Annexure 13.10: Institute ethical clearance (IEC) certificate

अखिल भारतीय आयुर्विज्ञान संस्थान, जोधपुर
All India Institute of Medical Sciences, Jodhpur
संस्थागत नैतिकता समिति
Institutional Ethics Committee

No. AIIMS/IEC/2021/3460 Date: 12/03/2021

ETHICAL CLEARANCE CERTIFICATE

Certificate Reference Number: **AIIMS/IEC/2021/3295**

Project title: **"Correlation of cytokine pattern in moderate to severe perinatal asphyxia with neurodevelopmental outcome: A prospective cohort study"**

Nature of Project:	**Research Project Submitted for Expedited Review**
Submitted as:	**D.M. Dissertation**
Student Name:	**Dr. Adil Ahmed Khan**
Guide:	**Dr. Arun Kumarendu Singh**
Co-Guide:	**Dr. Neeraj Gupta, Dr. Pratibha Singh & Dr. Mithu Banerjee**

Institutional Ethics Committee after thorough consideration accorded its approval on above project.

The investigator may therefore commence the research from the date of this certificate, using the reference number indicated above.

Please note that the AIIMS IEC must be informed immediately of:

- Any material change in the conditions or undertakings mentioned in the document.
- Any material breaches of ethical undertakings or events that impact upon the ethical conduct of the research.

The Principal Investigator must report to the AIIMS IEC in the prescribed format, where applicable, bi-annually, and at the end of the project, in respect of ethical compliance.

AIIMS IEC retains the right to withdraw or amend this if:

- Any unethical principle or practices are revealed or suspected
- Relevant information has been withheld or misrepresented

AIIMS IEC shall have an access to any information or data at any time during the course or after completion of the project.

Please Note that this approval will be rectified whenever it is possible to hold a meeting in person of the Institutional Ethics Committee. It is possible that the PI may be asked to give more clarifications or the Institutional Ethics Committee may withhold the project. The Institutional Ethics Committee is adopting this procedure due to COVID-19 (Corona Virus) situation.

If the Institutional Ethics Committee does not get back to you, this means your project has been cleared by the IEC.

On behalf of Ethics Committee, I wish you success in your research.

Dr. Praveen Sharma
Member Secretary
Member secretary
Institutional Ethics Committee
AIIMS,Jodhpur

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Appendix 13.11: Mastersheet