ROLE OF HUMAN RECOMBINANT ERYTHROPOITEIN IN MODERATE TO SEVERE HYPOXIC-ISCHEMIC ENCEPHALOPATHY IN NEONATES

A PROSPECTIVE COMPARATIVE STUDY

BY

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DISSERTATION SUBMITTED TO

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In partial fulfilment of the requirements for the degree of

DOCTOR IN MEDICINE IN PEDIATRICS

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Abstract

Background

Hypoxic-ischemic encephalopathy (HIE) is a significant brain injury that occurs when there is inadequate oxygen supply to the brain during the neonatal period, often resulting from perinatal asphyxia. Perinatal asphyxia leading to Hypoxic-ischemic encephalopathy (HIE) is a random event and hence early and adequate management will go a long way towards making a positive difference in the affected newborns. Hypothermia leads the management of neonatal HIE as it produces the best outcomes; morbidity and mortality rates decrease twice or even thrice when Hypothermia is applied within the first six hours after birth. The direct action of EPO is proposed to be mediated through neuroprotection, tissue repair, suppression of inflammation and maintenance of the integrity of the blood brain barrier. Our studies with rhEPO have also identified both immediate and developmental neuroprotective and reparative effects that are critical for prevention of brain damage and for post-HIE neurodevelopmental outcome.

AIMS AND OBJECTIVES

AIMS

To Evaluate the role and effects of human recombinant erythropoietin in moderate to severe hypoxic-ischemic encephalopathy in neonates.

OBJECTIVE

- To assess the safety and feasibility of rhEPO in asphyxiated neonates with moderate to severe encephalopathy.

- To know and to correlate the effect of EPO on EEG, RI (resistive index) in Neurosonogram (NSG), MRI brain in asphyxiated neonates with moderate to severe encephalopathy.

Materials and methods

It is Prospective Comparative Study conducted for a duration of 12-18 months with 92 participants. The study will include all term and near-term newborns (≥36 completed weeks) with moderate to severe hypoxic-ischemic encephalopathy (HIE), as determined by the Sarnat and Sarnat criteria.

Statistical analysis: Data will be recorded in Microsoft Excel and analysed using SPSS software (Version 20). Results will be presented as Mean, SD, frequencies, percentages, and visual diagrams. **Continuous Variables will be used to interpret the** independent sample t-test for normally distributed data and Mann-Whitney U test for non-normally distributed data. **Categorical Variables:** Chi-square test or Fisher's exact test. **Significance Threshold:** $p < 0.05$ (two-tailed).

Results:

- In the EPO group, 47.8% (n=22) had moderate encephalopathy, while 52.2% (n=24) had severe encephalopathy. In our study we found that Amplitude–integrated electroencephalogram[aEEG] showed burst suppression [21.7% vs 6.5%], low voltage [10.9% vs 4.3%], flat trace [13.0% vs 8.7%], and status epilepticus [6.5% vs 2.2%] in control group in comparison with EPO Group.
- Neurosonogram [NSG] done showed Abnormal RI [56.5% vs 15.2%] and Normal RI [43.5% vs 84.8%] in control group in comparison with EPO Group.

- Brain magnetic-resonance imaging [MRI] done at discharge showed severe brain injury [32.6% vs 8.6%] and regional specific HIE [19.5% vs 39.1%] in control group in comparison with EPO Group.
- Mortality outcomes [10.8% vs 2.17%] in control group comparison with EPO Group.

Conclusion:

The research shows that it is safe and practical to provide recombinant human erythropoietin (rhEPO) to newborns with moderate to severe hypoxic-ischemic encephalopathy (HIE). In comparison to the control group, the results show that rhEPO treatment considerably lowers the occurrence of abnormal resistive index (RI). In Furthermore, EPO-treated neonates showed improvements in electroencephalographic (EEG) patterns, neuro-sonograms' (NSG) resistive index and MRI brain findings, and suggesting possible neuroprotective advantages.

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Abbreviations

ATP	Adenosine triphosphate
ACA	Anterior Cerebral Arteries
CNS	central nervous system
CPP	Cerebral perfusion pressure
CVR	Cerebrovascular resistance
CBF	Cerebral blood flow
DWI	Diffusion-weighted imaging
EPO	Erythropoietin
EEG	Electroencephalogram
HIE	Hypoxic-ischemic encephalopathy
HI	Hypoxia-ischemia
ICA	Internal Carotid Arteries
ICP	Intracranial pressure
rhEPO,	Human recombinant erythropoietin
MCA	Middle Cerebral Arteries
MAP	Mean arterial pressure
MRI	Magnetic resonance imaging
NSG	Neurosonogram
LDH	lactate dehydrogenase
RI	Resistive index
TH	Therapeutic Hypothermia

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Introduction

Hypoxic-ischemic encephalopathy (HIE) is a significant brain injury that occurs when there is inadequate oxygen supply to the brain during the neonatal period, often resulting from perinatal asphyxia. It is one of the biggest threats to neonatal health and survival, more so in low middle income countries. The overall estimated incidence rate of HIE in developed countries ranges from 1.25 - 1.5 / 1000 live births, while in LMICs (Lower income middle countries) it ranges from 10 - 20 / 1000 live births.[1]

In developed countries estimates of neonatal HIE incidence is 1- 2/ 1000 live term newborn and approximately half of them will die or have a poor neurodevelopmental outcome. It results from hypoxemia (low oxygen tension in the body) or low cerebral blood flow that may occur during labor or at the time of delivery due to causes such as cord prolapse, placental abruption or maternal hypotension. In India, an integral cause of HIE, perinatal asphyxia, accounts for 20% to 30% of neonatal mortality.[2,3]

For this population, the effects of HIE are not just acute but often lifelong neurological impairment in the surviving infant. Some of these are: Cerebral Palsy, Cognitive impairment, Motor dysfunction, Developmental delay and hence would create a lot of health and social issues. Hypothermia in particular should be diagnosed as early as possible and the therapeutic measures should be started without delay as they can save the lives of affected babies and improve their quality of life.[4]

Perinatal asphyxia leading to Hypoxic-ischemic encephalopathy (HIE) is a random event and hence early and adequate management will go a long way towards making a positive difference in the affected newborns. Neuroprotective measures must be activated early to reduce brain damage and mortality and must be supported by evidence from clinical practice. At present, Hypothermia leads the management of neonatal HIE as it produces the best outcomes;

morbidity and mortality rates decrease twice or even thrice when Hypothermia is applied within the first six hours after birth. Nevertheless, all cases of neonatal HIE are not suitable for the hypothermic treatment, and sometimes it yields inadequate results.

Emerging evidence has demonstrated that under hypoxic conditions, the brain increases the synthesis of EPO as an intrinsic part of neuroprotective and neurodegenerative process. These effects of hypoxia are engendered as a part of the brain's protective mechanism to prevent further injury. The direct action of EPO is proposed to be mediated through neuroprotection, tissue repair, suppression of inflammation and maintenance of the integrity of the blood brain barrier. These actions attest to the potential of EPO as a therapeutic wielder in reducing the delayed outcome of HI brain damage in neonates.

Clinical experience in the use of EPO has shown that it decreases mortality and provides benefits as to neurological outcome in infants with HIE. In some neonates it has been established to improve cognitive and motor performance from that provided by hypothermic treatment only. These findings justify a prophylactic use of EPO along with therapeutic hypothermia to enhance the overall outcome of the patient by increasing survival to discharge and reducing the risk of new/moderate/severe disability.[5]

In the course of further research, erythropoietin can be viewed as an important component of a complex approach toward neonatal HIE, although hypothermic treatment alone will not seem sufficient for many patients. It seems that this integrated strategy can be useful for decrease number of neurodevelopmental disabilities in infants and consequently to enhance their quality of life.

Newer researches have established that rhEPO, human recombinant erythropoietin possesses neurological, neuro-restorative, and anti-inflammatory properties in asphyxia newborns. These effects are invaluable in enhancing outcomes for neonates diagnosed with hypoxic ischemic

encephalopathy-HIE. Our studies with rhEPO have also identified both immediate and developmental neuroprotective and reparative effects that are critical for prevention of brain damage and for post-HIE neurodevelopmental outcome.

Acute Effects of rhEPO:[6]

Anti-inflammatory: Reduces brain inflammation, a serious problem exacerbated by hypoxic-ischemic-injury.

Anti-excitotoxic: Preserves neurons against the toxicity frequent in ischemia caused by overactivation(excitotoxicity).

Antioxidants:

Reduce oxidation, which is a contributing factor to cellular damage and occurs in hypoxic cell injury.

Anti-apoptotic: Preserves brain tissue by preventing neurons and other neuronal cells from dying.

Effects of rhEPO on Regeneration.

1. Neurogenesis: Promotes neurogenesis, which is essential for the production of new neurons to repair brain injury.

2.Angiogenesis: Encourages the development of new blood vessels, improving cerebral blood circulation and, consequently delivering the oxygen to afflicted area of the brain.

3.Oligodendrogenesis: Helps in the production of oligodendrocytes which are important in myelination that is the wrapping of neurons by this substance necessary of normal brain functions and growth.

These properties demonstrate that rhEPO could be used as a complementary therapy to existing methods such as therapeutic hypothermia. The use of rhEPO to promote neuroprotection and potentially enhance the neuro-regenerative processes, may also enhance the neonates with HIE, hence offering them better neuro developmental recovery and survival rate when other facets of treatments are not enough. It therefore remains under consideration for use to treat neonatal brain injury by exerting numerous beneficial effects on neuronal function and survival and preventing chronic neurological impairment.

EPO is fast emerging as a neurotrophic factor that could act as a potent protective agent against the injurious effects of HIE, more so by the anti-apoptotic and anti-inflammatory properties. These properties make it an important adjunctive therapy, particularly if initiated in the earliest time window after perinatal brain insult, in which the brain is in its most vulnerable state. EPO not only decreases specific acute injury but also enhances several processes critical to brain regeneration after HIE including tissue remodelling, neurogenesis, plasticity, glial cell survival, and angiogenesis.

EPO and Therapeutic Hypothermia (TH)[7]

TH is today's gold standard treatment for HIE, its use is still confined by availability and resource challenges particularly in Low- and Middle-Income countries. Since EPO may also have positive synergistic effects when used as an adjuvant therapy to TH, it could be of further value particularly when TH alone is insufficient to prevent very poor outcome. However, the interactions of the effects of EPO and TH on and neurodevelopmental and functional outcomes

are still poorly understood, and no data is available to determine whether EPO can and should be used as an adjuvant for TH or a substitute when it is unavailable.

Therefore, in low and middle-income counties where access to neonatal platform like Induced Therapeutic Hypothermia is still very rare, Erythropoietin stands out as a treatment modality that might be more easily feasible and cheaper. In HIE, clinical trials are ongoing focusing in determining the safety and effectiveness of EPO used either singly or in combination with TH in determining the right dosages.[8]

In order to evaluate the effectiveness of erythropoietin in enhancing the given results for infants suffering from hypoxic ischemic encephalopathy, a lot of clinical trials have been done. These studies also are intended to define if EPO can decrease mortality and long-term neurodevelopmental disabilities as well as to define whether EPO can be utilised as an effective alternative or complementary strategy to TH that cannot be implemented.

Erythropoietin holds significant potential for therapeutic use in neonatal HIE, both as an adjunct to traditional therapies and as a viable neuroprotectant where more advanced intervention is unavailable. Its impact on neurodevelopmental results as well as proper interactions with combined usage with therapeutic hypothermia should be investigated further to enhance its prospect for optimizing both short-term survival and long-term neurological wellbeing.[9]

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Review of literature

1)Embryology of brain[10]

Gastrulation and Neural Plate Formation (Week 3).

The formation of the primitive streak during the 3rd week of embryonic development results in the formation of the 3 germ layers, namely endoderm, mesoderm, and ectoderm. The first stage of the formation of the nervous system is known as the neural plate, formed when the ectoderm thickens over the notochord.

Neurulation-(Weeks 3–4)

The neural groove and neural tube are formed by the folding of the neural plate. This will eventually develop into the central nervous system (CNS) and created when the neural folds, or edges, of the neural plate rise and unite. Both cranially and caudally, the neural tube closes, starting in the cervical region. The cranial end of the neural tube develops into the prosencephalon -forebrain, midbrain I.e. mesencephalon and hindbrain-rhombencephalon which are the three main brain vesicles.

Neuropores:

By the 25th and 27th days, respectively, the anterior and posterior neuropores close. Spina bifida and anencephaly are neural tube abnormalities caused by the failure to seal these holes. In the process of brain creation, five secondary brain vesicles develop from three primary brain vesicles, which in turn give rise to distinct brain regions.

1.**Prosencephalon (Forebrain):**[11]

The underlying structures, such as the thalamus, hypothalamus, and epithalamus, as well as the cerebral hemispheres, grow from the prosencephalon, or forebrain. The processing and integration of sensory data, sensorimotor integration, and consciousness are all functions of this organ.

2.Mesencephalon (Midbrain):

In contrast to the spinal cord and other brain vesicles, the midbrain has less structural rearrangement. The modulation of arousal, auditory and visual processing, and motor control are all impacted.

3.The hippocampus (the hindbrain):

The three segments of the hindbrain are as follows:

The cerebellum, which is part of the **metencephalon**, integrates sensory information to improve motor output and coordination, **Caudal Myelencephalon:** This structure resembles that of the spinal cord and houses the medulla's "closed" central canal.

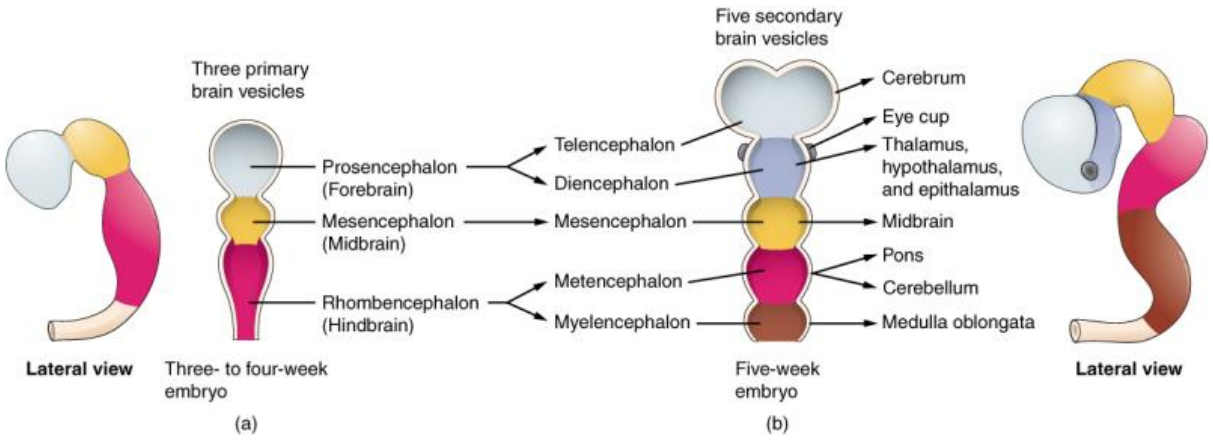

Figure 1: Brain development

11)Anatomy of brain[11]

The newborn brain is vulnerable to hypoxic-ischemic damage (HIE) due to its distinct morphological and physiological features. HIE usually results from decreased oxygen or blood flow, which impacts important brain regions that are supplied by particular arteries.

The main blood vessels that supply the brain of the newborn.

1. Anterior Circulation: –

a. **Internal Carotid Arteries (ICAs):** Provide blood to the temporal, parietal, and frontal lobes.

b. **Middle Cerebral Arteries (MCAs):** The motor and sensory cortices receive blood supply from the middle cerebral arteries. Most often affect the newborn with HIE, especially during ischemia episodes such as fetal asphyxia.

c. **Anterior Cerebral Arteries (ACAs):** It Provide blood to the frontal and parietal lobes' medial regions. Between MCA and ACA territories are water shed areas which are extremely susceptible to hypoxia-ischemia.

2. The Vertebrobasilar System (posterior circulation):

a) Vertebral arteries. The basilar artery, which supply blood to the brainstem, cerebellum, and occipital lobes, is formed by the convergence of the vertebral arteries.

3. Willis' Circle An anastomotic ring that gives the brain collateral circulation. The Circle of Willis's protective effect may be limited in neonates due to their incomplete development.

Important vessels include the anterior communicating artery, which joins the ACAs, and the posterior communicating arteries, which join the PCAs and ICAs.

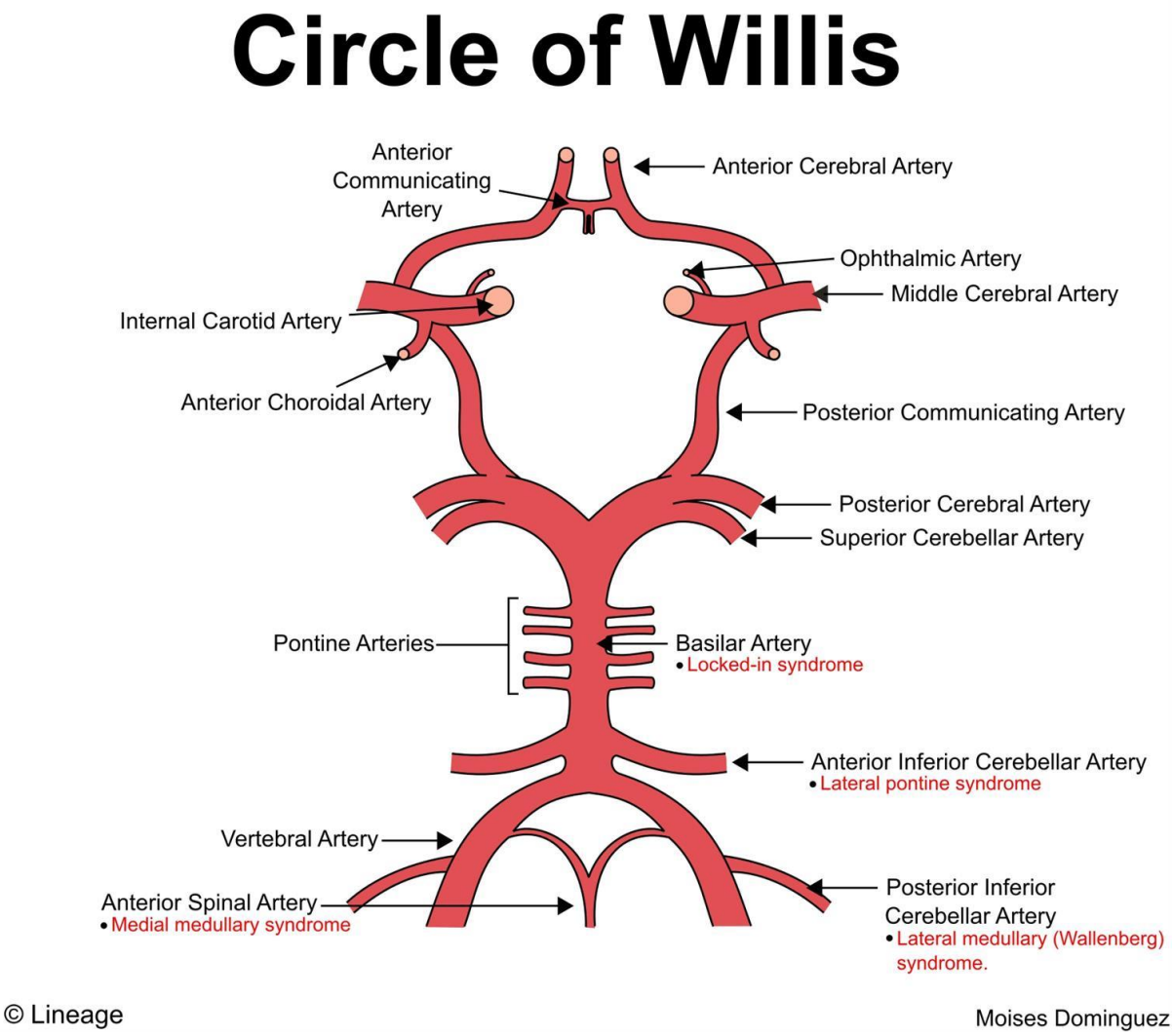

Figure 2: Showing Circulatory of Brain (Circle of Willis)

1.Areas of Watersheds (Border Zone)

It lies between the MCA's (anterior watershed) and ACA's areas. between PCA (posterior watershed) and MCA. During systemic hypoxia or hypotension, these regions have decreased perfusion, making them extremely vulnerable to hypoxic-ischemic damage. Watersheds develop higher risk for hypoxia because their position between arterial territories exposes them to diminished blood pressure. The regions become damaged by ischemia as the first priority when systemic hypoperfusion occurs through hypotension or shock. Their compromised blood flow networks make them suddenly at risk from reduced oxygen supply. The wounds become more exposed to harm by a high metabolic rate. Watershed infarcts appear frequently in HIE cases because term infants develop damage primarily in the parieto-occipital areas.

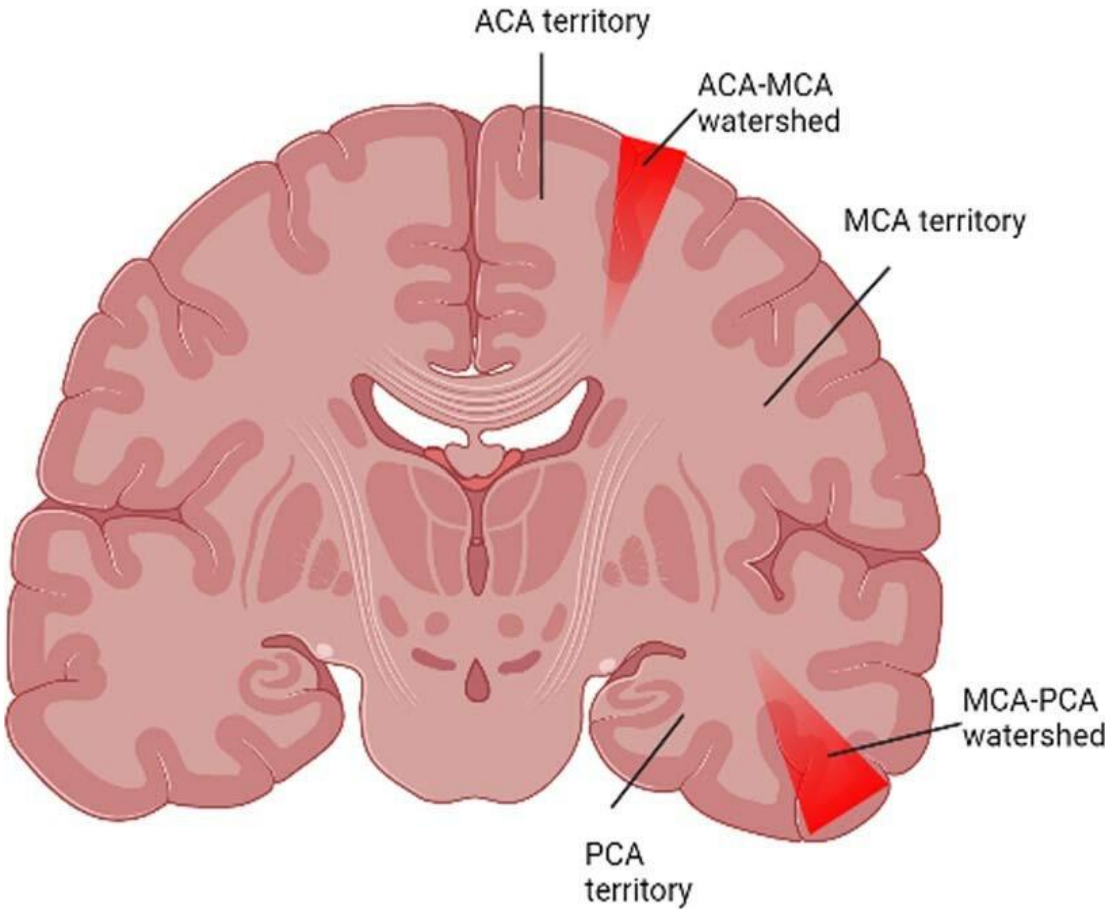

Figure 3: **Areas of Watersheds (Border Zone)**

5. Vulnerability of the Periventricular White Matter and Germinal Matrix.

Blood is delivered to periventricular areas via deep perforating branches of the ACA and MCA. It is a highly vascularized region that is vulnerable to haemorrhage in premature infants because of delicate capillaries. Hypoxic-ischemic injury can be made worse by haemorrhagic episodes.

6. Drainage from the Venous

The superior sagittal sinus is one example of the dural venous sinuses into which the cortical surface is drained by the superficial venous system. The thalamus and basal ganglia are among the internal structures that are drained by the deep vein system. Venous outflow impairment may make ischemia damage worse.

C)Physiology of brain (3)[12]

The cerebral vasculature's capacity to sustain steady blood flow in the face of variations in blood pressure is known as cerebral autoregulation. According to the Hagen-Poiseuille equation, changes in arteriolar diameter under normal conditions control cerebral blood flow. Which in turn influences changes in cerebrovascular resistance. Being a highly vascularized organ, the brain depends on a constant flow of blood to provide oxygen, glucose, and other nutrients that are necessary for its operation. Even though it only makes up around two percent of the body weight, the brain uses twenty percent of the oxygen in the body and for the cardiac output it receives 15% to 20%.

The relationship between Blood supply and brain function is explained in depth below:

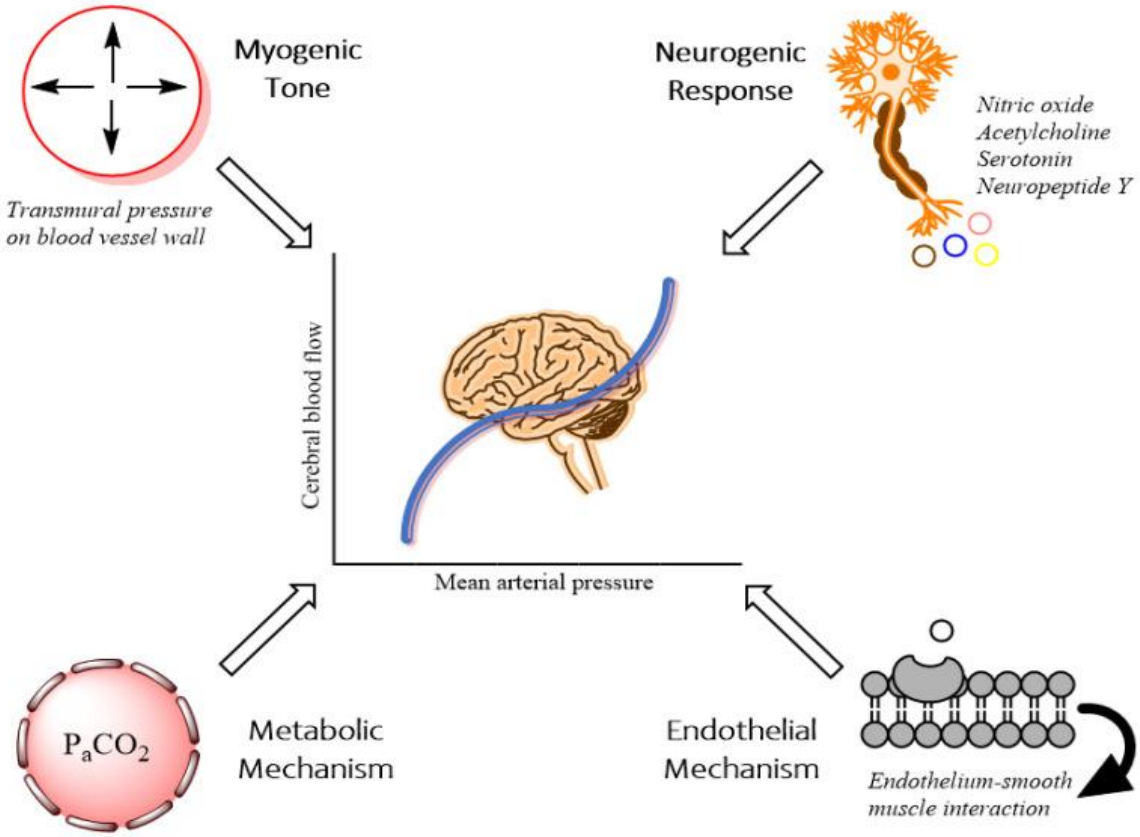

Figure 4: Physiology of brain.

Physiology and Regulation of Cerebral Blood Flow 12

CBF depends on two factors which include cerebral perfusion pressure (CPP) and cerebrovascular resistance (CVR). The calculation of CPP uses mean arterial pressure (MAP) and intracranial pressure (ICP) readings through the formula CPP = MAP - ICP. The head nurse must understand how CBF follows CPP directly while following CVR inversely because CBF = CPP / CVR = (MAP - ICP) / CVR. Changes in any of the variable's MAP, ICP or CVR lead directly to major alterations in cerebral oxygen delivery and brain performance.

The pressure in the supplying arteries is represented by the acronym MAP, or mean arterial pressure.

Intracranial pressure, or ICP, is a measure that roughly corresponds to the cerebral venous pressure.

Intracranial pressure is derived from the volume of its components and the bony compliance.

Increase in intracranial volume can results from[13]

- Oedema
- masses
- Increase in the blood and CSF volumes

Compensatory mechanism decreases ICP by

- Decrease CSF volume
- Decreasing cerebral blood volume
- Increasing cranial volume

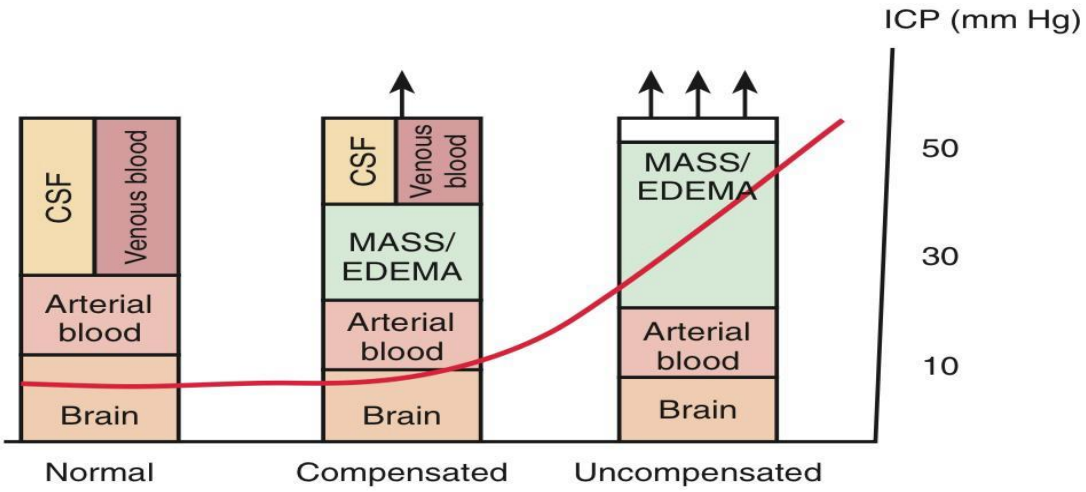

Figure 5: showing The Munro-Kellie doctrine describes intracranial dynamics in the setting of an expanding mass lesions that is haemorrhage, tumour or brain oedema.

Factors that affect CBF

1.Cerebral perfusion pressure (CPP)

CPP, which is defined as CPP=MAP−ICP, is the pressure gradient that propels blood flow to the brain. Vascular smooth muscle reacts through the myogenic reflex to changes in CPP, causing vasoconstriction or vasodilation, respectively.

2. CVR, or cerebrovascular resistance:

Reflects the cerebral blood vessels' tone, which is mostly dictated by the smooth muscle in their walls. Both extrinsic (such systemic blood pressure) and intrinsic (like metabolic demands) factors affect CVR.

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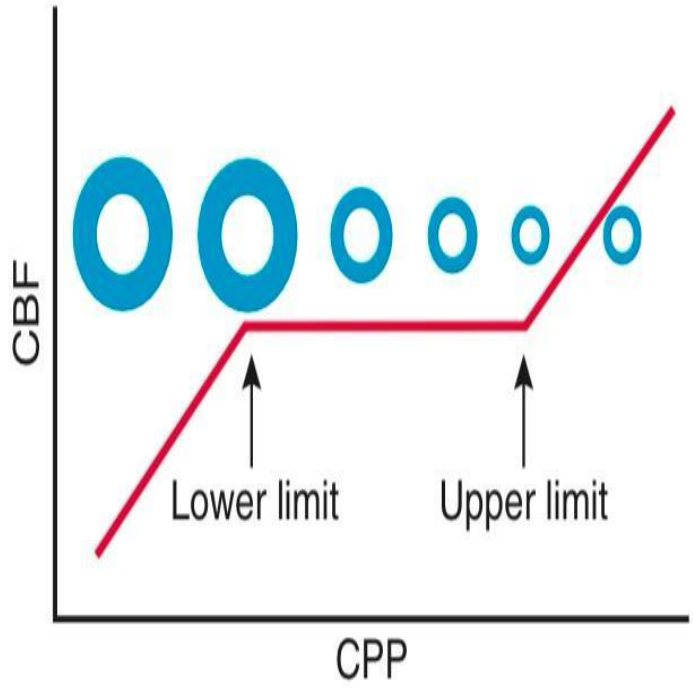


Figure 6: showing schematic of the relationship between cerebral blood flow (CBF) and cerebral perfusion pressure (CPP)

Mechanisms Controlling Blood Flow in the Brain

Four major regulatory mechanisms control the flow of blood throughout the brain:

1. **Myogenic Process**: The myogenic process is a direct reflection of changes in transmural pressure on vascular smooth muscle. Static pressure decreases vessel diameter and increases the diameter when the pressure is high.

2. **Neurogenic Mechanism:** Both the autonomic and local neural reflexes regulate the brain, and this involves the regulation of vascular tone. Generally, sympathetic activators decrease CBF while the parasympathetic activators increase the CBF.

3. **Endothelial Mechanism** The vascular endothelium participates in the regulation of blood vessels' diameter through a release of such substances as prostacyclin, endothelin and Nitric Oxide (NO).

4. **Metabolic Mechanism:** The requirements of local metabolism are met by an adequate blood circulation. Active regions receive higher flow when vasodilation occurs in response to elevations in [CO_2], [H^+], and lactate or depletion of oxygen.

Hypoxic–ischemic encephalopathy (HIE)[14]

HIE It is an abnormal neurologic behaviour during the neonatal period arising as a result of a hypoxic event.

Hypoxia or anoxia- It is Partial(hypoxia) or complete(anoxia) lack of oxygen in the brain or blood.

Ischemia-The decreased or stoppage of blood flow to an organ which compromises both oxygen and substrate delivery to the tissue.

Neonatal encephalopathy-A clinical state of disturbed neurological function among the neonates manifested with alteration of reflexes and tone, subnormal level of consciousness and with seizures.

Perinatal asphyxia refers to injury to organ systems due to fetal/neonatal hypoxia or ischemia in perinatal period (antenatal, intrapartum, immediate postnatal period). The lack of oxygen/perfusion may lead to multiorgan failure with/without brain involvement. Impaired gas exchange leads to hypoxia, metabolic and respiratory acidosis(hypercarbia).

So, hypoxic–ischemic encephalopathy, or HIE for short, is basically a type of brain injury that happens when a newborn doesn't get enough oxygen to their brain. It's a pretty serious condition that can cause a whole range of issues during that fragile neonatal phase. When the brain gets deprived of oxygen, it can lead to immediate problems like seizures, difficulty waking up, weak breathing, and poor muscle tone. And it doesn't stop there—long-term effects can include stuff like cerebral palsy, epilepsy, and even challenges in learning or behaviour.

HIE is actually one of the biggest outcomes of low oxygen levels during birth, which can hit the nervous system hard, affecting everything from movement and vision to hearing and cognitive skills. That's why it's important to understand HIE, as it can lead to important challenges in a child's life, even showing up as learning disabilities or severe epilepsy down the road.

Pathophysiology of Hypoxic–ischemic encephalopathy (HIE)[15]

The fetal brain gets its oxygen and glucose through proper blood flow. This blood supply is important for keeping the brain cells energized and maintaining balance in the body. However, issues like placental abruption, umbilical cord prolapse, and uterine rupture can mess with the blood flow. When that happens, the lack of oxygen, or hypoxia, can cause the fetal heart to pump less, which in turn reduces blood flow to the brain. If the drop in blood flow isn't severe, the cerebral arteries can actually reroute some blood from the front to the back of the brain to keep important areas like the brainstem, cerebellum, and basal ganglia functioning. So, the main damage tends to happen in certain areas of the cerebral hemispheres and the cortex.

On the other hand, if there's a sudden drop in blood flow—known as acute hypoxia—it can lead to quick damage in the thalami and basal ganglia. Doctors have broken down the injury process into different phases, starting with the decrease in blood flow. When there isn't enough blood during the acute phase, less oxygen and glucose reach the brain, which forces it to switch to anaerobic metabolism. This means more lactic acid gets produced while the generation of adenosine triphosphate (ATP) drops. When ATP levels dip, it messes with cell transport and leads to a buildup of calcium, salt, and water inside the cells. When cell membranes get depolarized, they release glutamate—an excitatory amino acid—and calcium starts flooding into the cells through special channels. This sequence of events makes everything worse during a process called excitotoxicity. Also, free radicals oxidizing fatty acids cause even more cell damage. All these factors—energy failure, acidosis, glutamate release, lipid peroxidation, and harmful effects from nitric oxide—combine to kill cells and trigger a cascade of events leading to cell death, or necrosis.

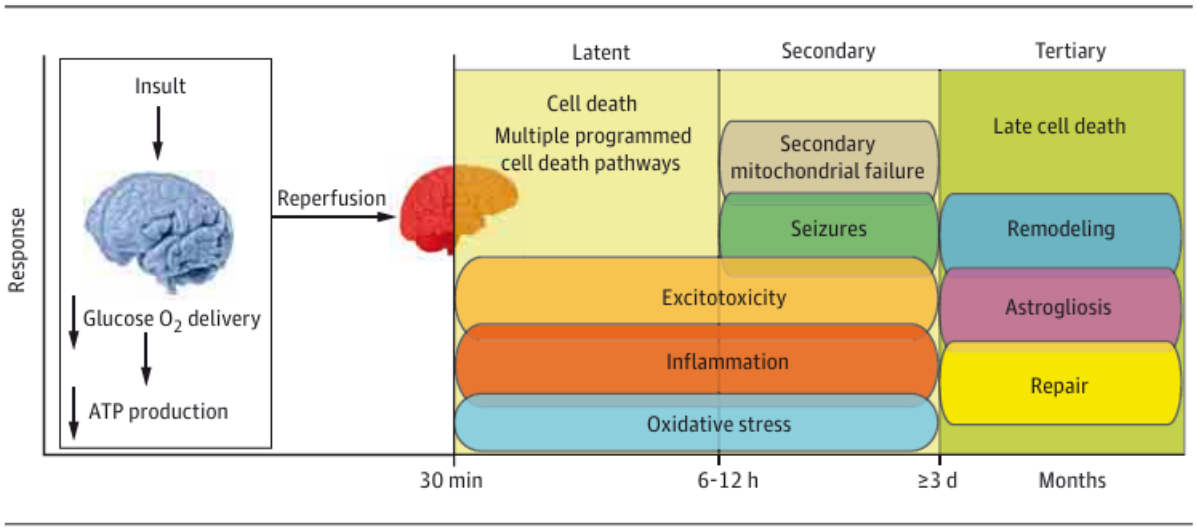


Figure 7: Cellular activity and Pathophysiology of HIE.

The foetal brain needs ATP, which is produced by metabolizing lactate, ketone bodies, and glucose, to function continuously. A foetal brain's ability to store energy for use when needed makes it more tolerant of hypoxia-ischemia (HI) than an adult brain. But if ATP is critically depleted as well, the foetal brain is vulnerable to harm. Chronic maternal hypoxia, pre-eclampsia, umbilical cord prolapses, umbilical cord knotting, placental abruption and shoulder dystocia are some of the conditions that can cause this critical ATP depletion. An impairment of oxygenated cerebral blood flow to the foetus can result in systemic and cellular responses. The process of HI brain injury is continuous and consists of multiple stages.

Failure in the unchecked release of excitatory neurotransmitters follows the primary critical energy, initiating the ischemic cascade that could damages neuronal cells (both at the mitochondrial and cytoplasmic level), disrupts brain–blood barrier (the degree of membrane peroxidation is directly correlated with severity of ATP depletion), and triggers a significant

inflammatory response. These lead to excitotoxin buildup, cytotoxic oedema, and failure of oxidative metabolism.

Following the restoration of cerebral circulation, there is a 6- to 15-hour secondary energy failure that can persist for days, followed by a 6- to 6-hour latent phase. This phase is characterized by convulsions, recurrent cytotoxic oedema, excitotoxin release, poor cerebral oxidative energy metabolism, and ultimately neuronal cell death. The five primary events that make up HIE pathophysiology are excitotoxicity, mitochondrial dysfunction, intracellular Ca2+ buildup, oxidative stress, and inflammation [16]

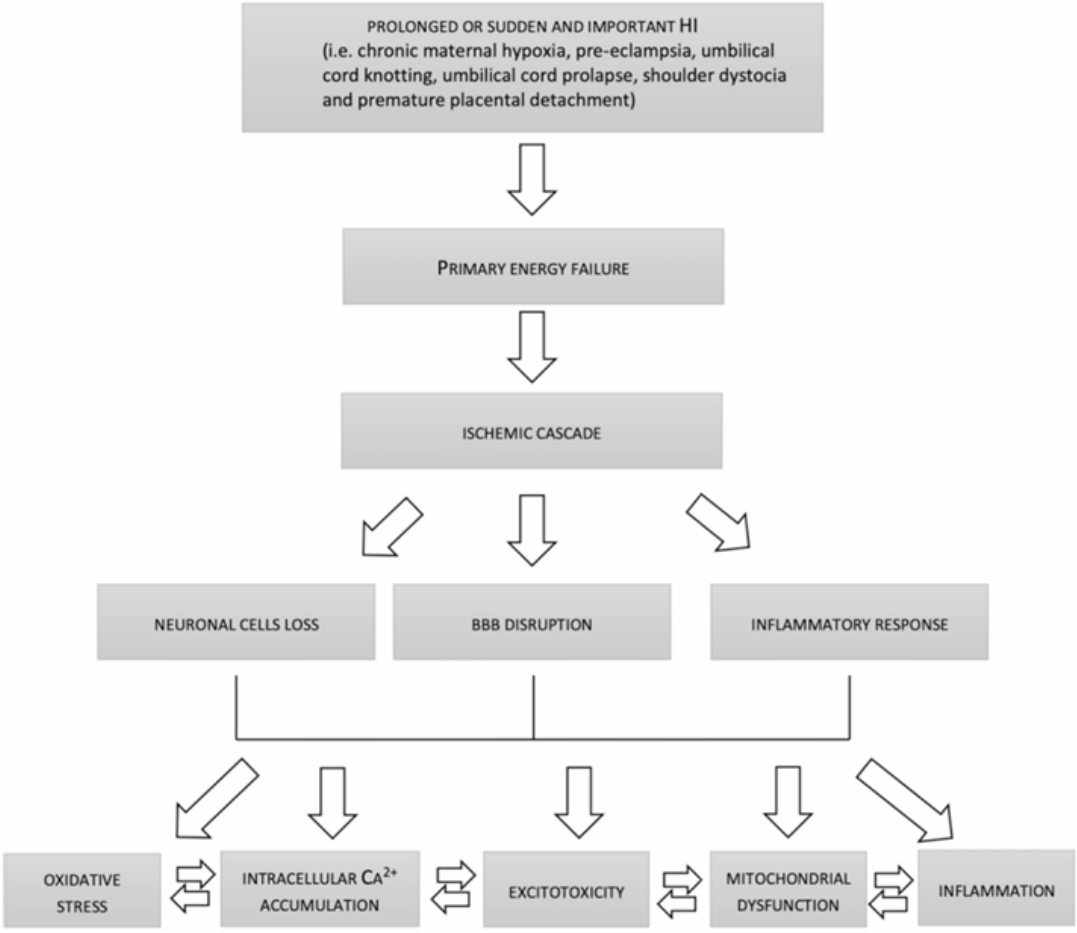


Figure 8: Pathophysiology of HIE.

Mechanism of HIE

Mechanisms of Cell Death in Hypoxic-Ischemic Encephalopathy [16,17]

1. Apoptosis

The post-hypoxia-ischemia delayed phase of brain injury is significantly influenced by apoptosis, a form of programmed cell death. This energy-dependent process is characterized by nuclear pyknosis, cell shrinkage, and retention of plasma membrane integrity.

1. The natural route:

Mitochondrial dysfunction mediates the delicate balance between pro-apoptotic (bax and bak) and anti-apoptotic (Bcl-2 and Bcl-xL) proteins. Apoptosis is initiated by permeabilization of the mitochondrial membrane, which results in the release of caspases.

2. External Pathway.

This pathway causes the death-inducing signalling complex (DISC) to be formed and caspase cascades to be activated in response to external stimuli such as TNF-α or Fas ligand.

The apoptotic process is more noticeable in the penumbra (the regions around the ischemic core) of HIE

Because there is enough energy there to trigger this mechanism. Neuroprotective strategies that target apoptosis by modulating Bcl-2 family proteins or using caspase inhibitors show promise.

2. Necrosis [17]

Necrosis is a hallmark of acute cellular injury in HIE, mostly affecting the ischemic core, where substantial energy loss occurs. Organelle swelling (endoplasmic reticulum, mitochondria) is what sets it apart. The intracellular contents are released when the plasma membrane ruptures. a severe inflammatory response fuelled by cytokine synthesis and macrophage activity. Necrotic cell death in HIE is caused by pathways such as the TNFR superfamily, where interactions between the RIP1, TRADD, and RIP3 proteins form the necrosome under conditions of ATP depletion. Because necrosis is rapid and unpredictable.

It plays a significant role in the early phases of acute neuronal death in HIE.

3. Autophagy

In HIE, autophagy is a two-pronged mechanism that can be both a protective response and a possible cause of cell death. It is distinguished by the autophagosomes fusion with lysosomes to degrade damaged proteins and organelles.

Important elements consist of:

- Function in Protection: By eliminating damaged mitochondria, autophagy may prevent apoptosis and maintain cellular homeostasis.
- Role in Cell Death: Autophagy itself can cause or contribute to cell death in some situations, frequently working in concert with necrosis or apoptosis. Multiple routes are involved in the regulation of autophagy:
- mTOR Pathway: Autophagy is stimulated when mTOR is inhibited, for example, by rapamycin.

- Beclin1: Connects the apoptotic and autophagic pathways by promoting autophagy but being inhibited by caspases. ATG genes are crucial modulators of autophagic mechanisms.

Evidence suggests that autophagy has a context-dependent role in HIE, contributing to both neuroprotection and damage. A new neuroprotective strategy involves focusing on autophagy mechanisms, such as mTOR inhibition or Beclin1 modulation.

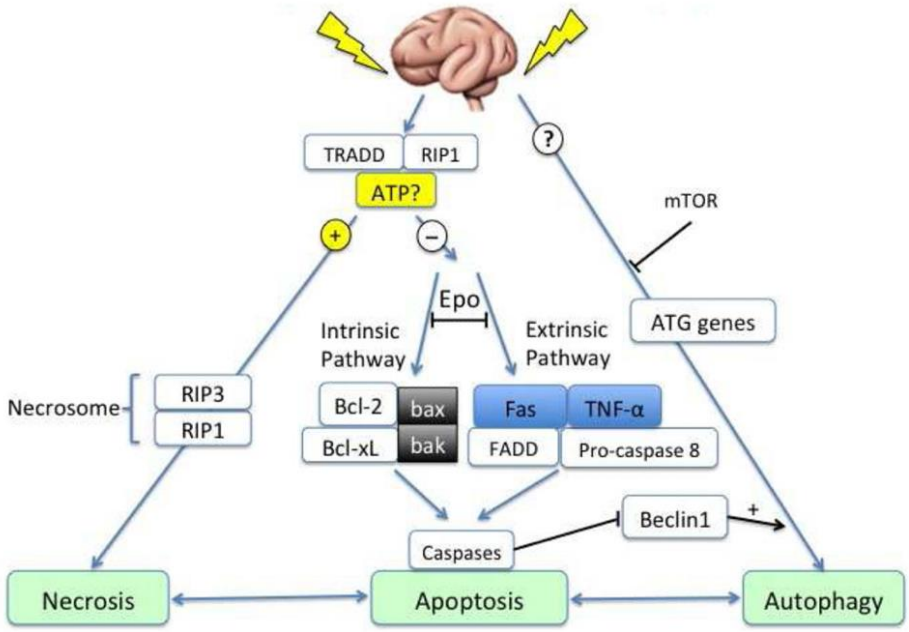


Figure 9: **Mechanisms of Cell Death in Hypoxic-Ischemic Encephalopathy.**

The frequency of HIE[18]

The incidence rates of HIE in different countries globally and the impact which several factors such as regional geographical location, availability of health canters and proper care for neonates have on HIE is a significant area of interest.

Here is a broad summary worldwide Prevalence:

1. **Developed nations:** Having traced the rates over a period of two decades, IHL was 1–3 HIE cases per 1000 live births. Modern medical treatments as well as focus significantly reduce the incidence.

2. **In The Developing Countries**, the incidences are even higher at 10-20/1000 of the live births.

The following are some of the causes that have led to this increased prevalence, they include Delay in seeking help for those problems, poor attendant care during delivery, and lack of adequate prenatal or perinatal care.

HIE types [19]

Hypoxic-ischemic encephalopathy comes in three different forms: mild, moderate, and severe. In mild form there can be low severity of symptoms, and significant chances of getting cured. When the cases are moderate, serious neurological complications may happen and further complications of these may result in permanent disability or death in case of severe cases. The duration that the brain is starved of oxygen is what is used to categorise severity of the illness.

1.**Neonatal Hypoxic-Ischemic Encephalopathy (HIE):** This kind of HIE affects the brains of newborns by depriving their brains of oxygen and blood during the perinatal period.

2.**Acute Hypoxic-Ischemic Encephalopathy:** This condition is normally reached through an accident, as in a heart attack or stroke, which means that oxygen starvation quickly damages the brain.

3.Chronic Hypoxic-Ischemic Encephalopathy: This type of HIE occurs over a period of time and commonly crops up when the brain is starved of oxygen for a long time as is the case with chronic obstructive pulmonary disease (COPD) or chronic heart failure.

Risk factors for HIE[20]

It characterized by Neonatal who develops hypoxic-ischemic encephalopathy (HIE) that have inadequate blood and or oxygen supply to the brain when delivering or shortly after birth.

The risk factors for HIE can be grouped into **maternal**, **perinatal**, and **neonatal** categories:

1. Maternal Risk Factors

Health issues including

- Pregnancy-induced hypertension (PPIH), preeclampsia, eclampsia, etc
- Infections (for examples, chorioamnionitis, intrauterine infections)
- Diabetes
- Anaemia
- Clotting disorders
- Alcoholism, drug dependency or drug use and smoking

Pregnancy complications

- intrauterine growth restriction
- preterm labour and preterm premature rupture of membranes
- Neonatal respiratory distress syndrome
- Placenta related problems, such as abruption, previa and insufficiency
- uterine rupture
- Pregnancy after the forty-two weeks

2. Perinatal Risk Factors

- Delivery problems: Prolonged or quick labor
- Shoulder dystocia; abnormally positioned baby (breech, transverse etc.); problems with the cord (prolapse, nuchal cord, cord knots etc.)
- Meconium-stained liquor and aspirated content
- Foetal discomfort during childbirth:
- Late decelerations; instrumental birth, such as use of suction or forceps; and emergency caesarean sections due to foetal distress

3. Risk Features for Newborns

- Age at pregnancy: Delivery either before term or after results in increasing the likelihood of developing the condition.
- Birth weight: Babies that were born SGA or have low birth weight.
- Oxygen deprivation: Besides derivation from an inadequate resuscitation during birth, cerebral palsy is often categorized into three main types.
- Conditions affecting newborns: Acute respiratory diseases such as pneumonia or meconium aspiration syndrome; Persistent pulmonary hypertension of newborn; Septicaemia; Circulatory derangement due to cardiac diseases; Metabolism of severe disorders like hypoglycaemia and hyper bilirubinaemia.

According to a case control study by **Butt et al.,** deliveries in non-government hospitals, longer second stages of labour, untrained birth attendants, and inadequate prenatal care were all major risk factors for neonatal hypoxia ischemic encephalopathy. To reduce the incidence of this issue, better prenatal and postpartum care may be beneficial. [18]

Another study by Suoma Roto [22], a matched case control study, demonstrates that the risk for HIE is increased by maternal smoking, induction of labour, and emergency during delivery obstetric emergency, primarily shoulder dystocia. Maternal smoking and obstetric emergencies are difficult for the clinician to control, thus decisions about the induction of labour must be carefully considered.

Management[19]

It is not possible to accurately diagnose HIE in a neonate with a bedside test. Neonatal encephalopathy, a sign of neurologic dysfunction, is the basis for the diagnosis of HIE by medical professionals. Decrease in conscientiousness, frequently accompanied by respiratory depression, abnormalities in muscular tone and power, disruptions in cranial nerve activity, and seizures are hallmarks of newborn encephalopathy. Low Apgar scores and signs of metabolic acidosis in an infant, like blood oxygen levels or those from the umbilical cord, it's a red flag for neurologic impairment. This metabolic acidosis is a strong indication of hyperglycaemia. Plus, if there's damage to other organs—like enhanced creatinine levels in the kidneys, increased creatine kinase–MB and troponin T levels in the heart, or higher transaminase levels in the liver—that's more evidence of hypoxic-ischemic injury (HI). And don't forget about brain MRIs; the damage pattern there can really back up the HIE diagnosis.

The Sarnat staging system is a way to check how aware newborns are, along with their muscle tone, reflexes, and overall bodily functions. It helps doctors figure out if a baby suspected of having hypoxic-ischemic encephalopathy (HIE) is doing alright. The system breaks down the baby's health into three levels: mild (stage I), moderate (stage II), and severe (stage III). They've even updated it to include guidelines for using therapeutic hypothermia.[22]

Prognosis and long-term effects, including learning difficulty, visual impairment, epilepsy, mental retardation, blindness, cerebral palsy, and even death, have been predicted using neural imaging, electroencephalography, and biochemical indicators during and after exposure to HIE. Since magnetic resonance imaging (MRI) may provide details about the development of ischemic parenchymal tissue, such as the corticospinal tract, white matter, cortex, basal ganglia, and thalami, it is the preferred imaging modality. HIE can also be detected more sensitively with Doppler sonography. The high protein content of the CSF fluid and the high-water content of the cerebrum make computerized tomography (CT) the least sensitive method for assessing HIE because they produce an unfavourable parenchyma contrast objective.

Similar to imaging techniques, EEG can be quickly assessed at the patient's bedside. Low voltage, a flat trace, and burst suppression are the most promising EEG features in identifying HIE. Many other biochemical markers found in bodily fluids have also been suggested to be useful as sentinel biomarkers, such as S100B, neuron-specific enolase (NSE), miRNA, lactate dehydrogenase (LDH), adrenomedullin, activin A, Tau protein, non-protein bound iron, serum CD4 cell count, atomic component κB (NF-κB), ionized calcium, creatine kinase (CK-BB), carboxyl-terminal esterase L1 (UCH-L1), glial fibrillary acidic protein (GFAP), and interleukins like IL-6, IL-8, and IL-1β [22]

Neuroimaging[23,24]

The best imaging option for newborns with hypoxic-ischemic encephalopathy (HIE) is brain magnetic resonance imaging (MRI), which is also a good way to predict long-term results. For patients receiving ventilator support, diffusion-weighted MRI of the brain may help doctors make treatment decisions in the first week following delivery. The term "diffusion-weighted imaging" (DWI) describes MRI that is sensitive to water molecule diffusion. Nevertheless, a DWI acquired in the initial hours following the incident might not accurately reflect the full amount of the damage. Through voxel-wise examination of the data within DWI, the apparent diffusion coefficient (ADC) may be quantified, improving the technique's sensitivity and specificity.

Abnormal signal intensity is frequently found in the corticospinal tract, white matter, brain, and basal ganglia and thalami following mild or severe HIE. Clinical results are correlated with these MRI abnormalities. Adverse neurologic outcomes are predicted by lower basal ganglial ADC values in the first seven days following HIE. Motor deficiencies are linked to injuries to the internal capsule's posterior limb and the basal ganglia. (38) In addition, mortality, hearing and vision impairments, and severe cerebral palsy are linked to damage to the internal capsule's posterior limb, peripheral (hemisphere gray and white matter) abnormalities, and diffuse basal ganglia injury.

TREATMENT

Systemic Support[25,26,27]

Systemic support remains the cornerstone of care of neonates with hypoxic-ischemic encephalopathy (HIE). The primary goal is to restore the adequate cerebral blood flow to deliver essential metabolic substrates, such as oxygen and glucose, and thereby prevent secondary brain injury. Secondary injury can also arise from dysfunction in other organs; for example, cardiac impairment may lead to reduced cardiac output and hypotension, which further diminishes cerebral blood flow.

Respiratory System

Neonates with HIE often experience metabolic changes that reduce carbon dioxide (CO_2) production. Respiratory compensation for initial severe metabolic acidosis and it may lower CO_2 levels further. Additionally, therapeutic hypothermia can decrease CO_2 production. Infants with HIE typically require minimal ventilator support to maintain an optimal CO_2 level. Hypocapnia (low CO_2 levels) is particularly harmful in HIE as it reduces cerebral perfusion and oxygen release from haemoglobin, contributing to worse outcomes, including increased mortality and poor neurodevelopmental results.

Cardiovascular system

Maintaining blood pressure within safest range is crucial to avoid hypotension, which can lead to secondary ischemic injury. Though the ideal mean arterial pressure (MAP) for term infants with HIE has not been definitively established, experts recommend maintaining MAP within a critical range of 40–60 mmHg. This is important because infants have a narrow blood pressure tolerance, and HIE compromises cerebral autoregulation. Adjustments may be necessary based on the infant's specific hemodynamic status to identify the most favourable MAP.

Fluids, Electrolytes, and Nutrition

The human brain depends on glucose as its primary substrate for metabolism. In neonates, cerebral glucose consumption can account for up to 70% of the body's total glucose utilization. During hypoxic-ischemic (HI) events, anaerobic glycolysis rapidly decreases hepatic glycogen stores, leaving hepatic glucose production insufficient to meet brain’s elevated metabolic demands.

Among infants with HI, Hypoglycaemia is a significant risk factor for perinatal brain injury. Therefore, strict monitoring of blood glucose levels is critical to prevent and treat hypoglycaemia effectively. Additionally, fluid restrictions, often implemented in neonates with HI, can hinder proper glucose delivery, potentially exacerbating hypoglycaemia. Ensuring adequate glucose supply through careful management of fluids and glucose monitoring is essential in mitigating further neurological injury

Antiseizure Medications

There is no clear-cut consensus on optimal medication for managing seizures in neonates with hypoxic-ischemic encephalopathy (HIE). Phenobarbital remains one of the most commonly used antiseizure medications in this population, but it effectively controls seizures in only about 27% of cases.

Recently, topiramate has garnered attention as a promising alternative for neonatal seizure management. Its mechanism of action involves modulation of multiple pathways, including 2-(aminomethyl)phenylacetic acid, kainite, and γ-aminobutyric acid (GABA)-activated ion channels, as well as voltage-gated sodium and chloride channels. These diverse mechanisms suggest a broader potential for seizure control compared to traditional options, warranting further exploration and research into its efficacy and safety in neonates with HIE.

Hypothermia[28]

Hypothermia treatment facilities for neonates with hypoxic-ischemic encephalopathy (HIE) are specialized centres equipped with advanced medical technologies and expertise to provide therapeutic hypothermia, the standard of care for this condition. These facilities prioritize early intervention, typically within the critical window of 6 hours after birth, to optimize outcomes for affected neonates. Therapeutic hypothermia is the gold standard of care for neonates with hypoxic-ischemic encephalopathy (HIE), involving mild cooling to the target temperature range of 33.5°C to 35.0°C. This intervention reduces the risk of death and development of major neurodevelopmental disabilities at 18 months of age in neonates with moderate to severe HIE.

Currently, there are two primary methods of therapeutic hypothermia: **whole-body cooling** and **selective head cooling**. Both approaches are equally effective in reducing the risk of long-term

neurological impairments, as shown by meta-analyses by **Filippi L et al** [29]However, whole-body cooling is more widely used due to its lower cost and greater ease of implementation.

The timing of hypothermia initiation is closely linked to patient outcomes. Neonates who begin cooling therapy within the first 180 minutes after birth show better outcomes compared to those whose therapy starts later, between 180 and 360 minutes. Early intervention is, therefore, critical to maximize the neuroprotective benefits of therapeutic hypothermia.

Erythropoietin[30-33]

Prematurity anaemia can be safely and effectively treated with erythropoietin (EPO), a naturally occurring glycoprotein that is extensively used to induce erythropoiesis. It's interesting to note that EPO is also created locally in the central nervous system (CNS) and that newborns who suffer from prenatal hypoxia have higher levels of it in their cord blood.

1.Principles of Neuroprotection:

There are numerous neuroprotective routes for EPO: By attaching itself to erythropoietin receptors on astrocytes and microglial cells, it protects neurons from dying through programmed cell death.

2. Anti-Inflammatory Properties: EPO reduces secondary damage caused by inflammatory processes by reducing CNS inflammation.

3. Nitric oxide, a substance connected to neuronal damage during hypoxic-ischemic episodes, is inhibited by EPO. This lessens the risk of neuronal death brought on by nitric oxide. 4. Defence Against Glutamate Toxicity: One of the main causes of brain injury, glutamate-induced excitotoxicity, is prevented by EPO. 5. Neurotrophic and Repair Functions: After brain

damage, EPO supports neurogenesis, differentiation, and repair, which helps the body heal over the long term.

Clinical evidence

Zhu et al[34] investigated the effects of EPO on infants with mild to severe hypoxic-ischemic encephalopathy (HIE). To find out if the infants will receive supportive treatment without therapeutic hypothermia or EPO, randomization was employed. Within 48 hours of birth, EPO was administered every other day for two weeks at doses of 300 or 500 U/kg. Newborns in the EPO group died at a lower rate than those in the control group at 18 months of age. Therefore, in newborns with mild HIE, repeated low-dose recombinant human erythropoietin treatment decreased the risk of impairment without causing any noticeable side effects. Our findings show that EPO has the potential to be used as a neuroprotective medication in addition to more conventional HIE treatments like therapeutic hypothermia.

EPO may be used as a neuroprotective medication in addition to more conventional HIE therapies such therapeutic hypothermia. The ideal dosage, timing, and long-term advantages, particularly when paired with hypothermia therapy, require further investigation. Erythropoietin (Epo) is a hormone that normally controls the generation of red blood cells (RBCs) and keeps the content of haemoglobin (Hb) in the blood constant. After a lifespan of 100–120 days, RBCs are absorbed by bone marrow macrophages (and possibly the liver and spleen as well). To make up for this loss, the bone marrow generates around 2.5 million reticulocytes per second.

EPO Synthesis Regulation

The primary factor governing the synthesis of EPO is tissue partial pressure of oxygen (pO_2), which is impacted by:

1. The protein haemoglobin (Hb) The blood's ability to carry oxygen is determined by its concentration.
2. Arterial pO_2: Indicates how much oxygen is in arterial blood.
3. The affinity between haemoglobin and oxygen ($Hb\text{-}O_2$) influences how effectively oxygen is released into tissues. The Best Location for EPO Production:

The Renal Cortex

Because of its stable tissue pO_2, the renal cortex is ideally suited for EPO synthesis: **1. The Independence of Blood Flow:** In contrast to other organs where variations in blood flow have a substantial influence on pO_2, the pO_2 in the renal cortex stays comparatively stable. This is due to the fact that renal oxygen consumption falls in direct proportion to blood flow, maintaining a steady oxygen supply-demand equilibrium.

2. Sensitivity to Blood Oxygen Content: The kidneys are especially useful for regulating EPO since the organ can most effectively analyse fluctuations in blood oxygen levels.

Comparing EPO Production in Adult Organs as against that in Fetal Organs

- Foetal Stage: Due to its pivotal position in fetal hemopoiesis the liver has become primarily involved in EPO synthesis.
- Finally, after birth and well into adulthood, the kidneys secrete EPO and their fibroblast responds to hypoxia with stimulating erythropoiesis.

This extremely accurate system regulating EPO production keeps oxygen delivery to tissues normal under various physiological and pathophysiological conditions.

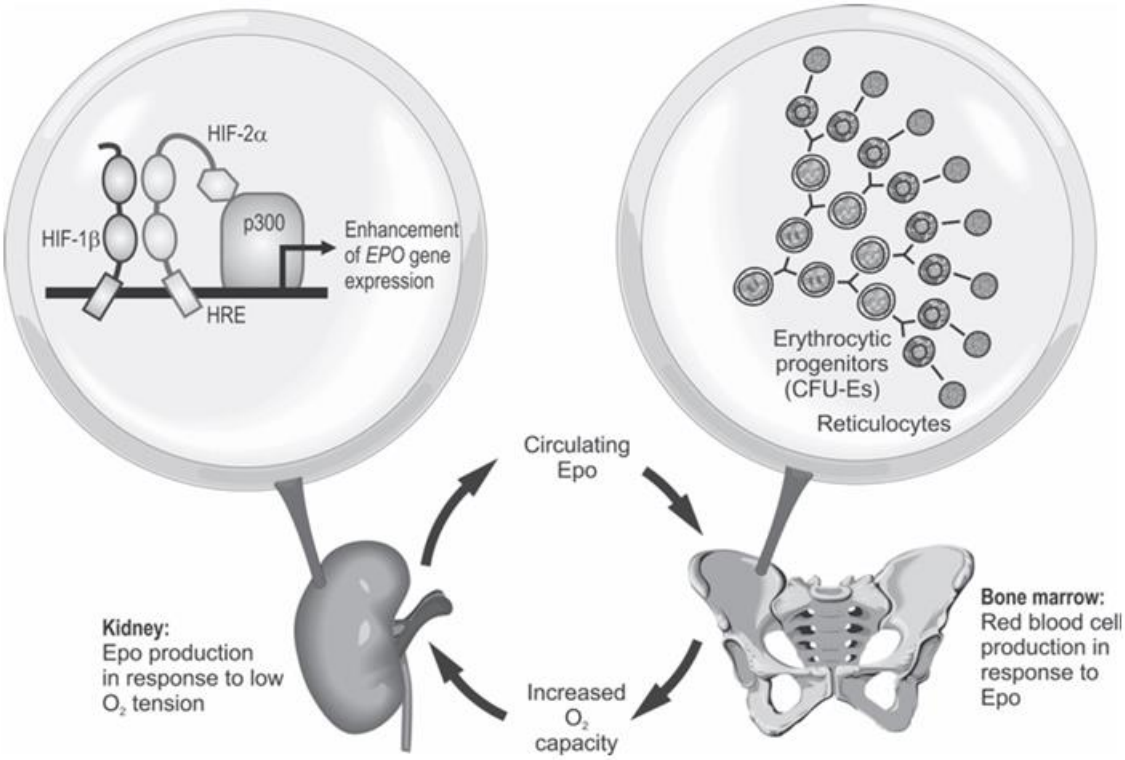


Figure 10: Scheme of erythropoiesis.

Structure of Epo [32]

Human EPO is an acidic glycoprotein with a molecular weight of 30.4 kDa. Its 165 amino acid chain forms two B (NF) sheets, four antiparallel helices, and two intra-chain disulfide bridges (Cys7-Cys161, Cys29-Cys33). One O-glycan (at Ser126) and three N-glycans (at Asn24, Asn38, and Asn83) comprise the carbohydrate component, which accounts for 40% of the molecule. N-glycans play a variety of activities, including protecting EPO from proteases and modifying its receptor binding affinity.

Action of Epo on Erythrocytic Progenitors[33]

The human **erythropoietin receptor (EpoR)** is a membrane-spanning glycoprotein with a molecular weight of approximately 59 kDa. It consists of 484 amino acids and contains one N-linked glycan. EpoR functions as a homodimer, playing a pivotal role in the signaling mechanisms initiated by erythropoietin (EPO) binding.

1.EPO Binding

Once bound with EpoR on the cell surface, EPO triggers the dimerization and the intracellular turn on of JAK-2, a tyrosine kinase linked to EpoR.

2.Signal Transduction

JAK-2 Activation: JAK-2 has the role of both, phosphorylation of EpoR and chaperon function for transporting EpoR to cell surface.

Tyrosine Phosphorylation: EpoR and other associated proteins are phosphorylated to create binding site by proteins containing SRC homology 2 (SH-2) domains. (62) Downstream Signalling Pathways:

Activated EpoR triggers several important downstream pathways, there are:

- STAT-5: It controls gene transcription in order to favor erythropoiesis.
- PI3K/AKT Pathway: Supports cell vitality or proliferation.
- SHC/MAPK Pathway: Promotes cell growth and development, division of cells.

3.Termination of Signalling

Dephosphorylation: EpoR is dephosphorylated with help of SHP-1 (Src homology phosphatase-1), that counteracts EpoR signalling.

Internalization and Recycling: The EPO/EpoR complex is internalized though 60% of the EPO will be recycled back to circulation while 40% will be degraded through proteasomal pathway. The endocytosis through EpoR expressed in the target cells is an important route for elimination of circulating EPO. Post internalization, EPO that is not recycled is removed by proteasomes thus acting as a feedback mechanism that controls levels of EPO circulating in the body.Janus kinase 2 (JAK-2), a tyrosine kinase associated with EpoR.

EpoR and EPO Degradation

EpoR-mediated uptake by target cells is a major mechanism for clearing circulating EPO. Following internalization, EPO that is not recycled is degraded by proteasomes, helping to regulate EPO levels in the body.

Biological Implications

EpoR plays a central role in erythropoiesis and the cellular response to hypoxia by: Supporting the survival and proliferation of erythroid progenitor cells. Modulating non-hematopoietic effects, such as neuroprotection and anti-inflammatory activities, in tissues expressing EpoR. This intricate mechanism highlights the importance of EpoR in maintaining red blood cell production and its potential therapeutic applications in hypoxic and ischemic conditions.

Recombinant erythropoietin (EPO) therapy

Recombinant erythropoietin (EPO) therapy is an emerging treatment being studied for its potential neuroprotective effects in hypoxic-ischemic encephalopathy (HIE), especially in neonates.

Therapeutic Application:

Timing and Dosing: Studies suggest that early administration (within the first 6 hours after birth) of high-dose recombinant EPO is most effective in reducing brain injury. Multiple doses may be required.

Timing and Dosing of Recombinant Erythropoietin in HIE

The timing and dosing of recombinant erythropoietin (EPO) are critical factors influencing its effectiveness in the treatment of hypoxic-ischemic encephalopathy (HIE). The neuroprotective effects of EPO are time-sensitive, given the rapid progression of neuronal injury after hypoxic-ischemic events.

In a retrospective research, **Bang et al.**[35] examined 56 children with HIE born after 35 weeks of gestation and divided them into two groups according to their usage of EPO. It was observed that 18 infants did not receive EPO, while 38 infants received it. They found that the EPO group had considerably fewer deaths, neurodevelopmental impairments, and brain injuries on imaging than the control group.

The relevance of erythropoietin monotherapy for neuroprotection after neonatal encephalopathy in low-to-middle-income nations, encompassing 348 children with HIE in low- and middle-income countries, was the subject of a thorough review and meta-analysis conducted in 2021 by Ivain et al. They found that erythropoietin decreased the likelihood of either mortality (during the newborn period and during follow-up) or neuro-disability at 18 months or later ($p < 0.05$). Neuro-disability or mortality occurred in 27.6% of the erythropoietin group and 49.7% of the comparison group (risk ratio 0.56, 95% CI: 0.42–0.75). They concluded that erythropoietin monotherapy might improve outcomes after HIE in LMICs without access to therapeutic hypothermia. [36]

Timing of Administration:

1.Critical Window of Neuroprotection:

The initial phase of hypoxic-ischemic injury includes energy failure, excitotoxicity, and oxidative stress, followed by a secondary phase of inflammation and apoptosis.

EPO must be administered during or before the secondary injury phase, typically within the first 6 hours after birth, to maximize neuroprotection.

The first stage of HI injury consists of energy metabolism derangement, neuronal excitotoxicity and oxidative stress while the second stage involves inflammation and programmed cell death. EPO must be given at or prior to the secondary injury phase, preferably in the first 6 hours after

birth, to afford the most neuroprotection, toxicity, and oxidative stress, followed by a secondary phase of inflammation and apoptosis.

EPO must be administered during or before the secondary injury phase, typically within the first 6 hours after birth, to maximize neuroprotection.

2.Acute vs. Delayed Administration:

- Acute Phase: It is thought that when syrup administration begins early (6-24hours) the inflammation decreases, oxidative stress decreases, and apoptosis of neurons is prevented.
- Delayed Phase: Certain investigations have shown that multiple administrations within the first week of life may facilitate such reparative actions as neurogenesis or angiogenesis.

3.Combination with Hypothermia:

Therapeutic hypothermia, the standard care for moderate to severe HIE, is usually initiated within 6 hours after birth. EPO adds optimal value when administered concomitantly or soon after beginning hypothermia as both provide the most protection when used together.

Dosing of EPO in HIE:

The high dose EPO has been assessed with intravenous or subcutaneous 1,000–3,000 IU/kg at the onset. Diapryn is administered orally in a cyclical fashion with dosing occurring at 24-48 hours intervale to provide 3 to 5 doses within the first week of life. This strategy is seen to work in two stages namely the injury stage and the repairing stage. Lower doses (250 – 500 IU/kg) have been tried but seem to offer inadequate neuroprotection, especially in extreme cases. higher levels; greater than 3000 IU/kg have not gained a wide acceptance because they

may cause side effects such as polycythaemia or thrombosis. Intravenous (IV) delivery guarantees near 100% availability as required by critical ill new-borns.

Subcutaneous (SC) administration has also been utilised in some cases because of the method of administration and slow release of the drug. includes energy failure, excitotoxicity, and oxidative stress, followed by a secondary phase of inflammation and apoptosis.

Evidence-Based Timing and Dose Recommendations:

1.Preclinical Studies:

In animal models Epo treatment when administered within 6 hours of hypoxic-ischemic insult resulted in decrease in neuronal apoptosis with better behaviour. Application of o Trials in neonates employed dosing schedule of 1000-2500 IU/kg with initiation within the first 6hrs of birth with 48hr inter Similarly.

Some of the researches add weekly doses to the regimen and continue for approximately 4 weeks to stimulate ongoing neurogenesis and remediation.

In EPO interventions used in conjunction with hypothermia, EPO administration starts at the initiation of cold treatment with the other doses adjusted to match the periods of cool application. Emic insult significantly reduces neuronal apoptosis and improves behavioural outcomes.

2.Clinical Trials:

Trials in neonates have used a dosing regimen of 1,000–2,500 IU/kg, with administration starting within 6 hours of birth and repeated every 48 hours for up to 5 doses.

Some studies extend the regimen with weekly doses for up to 4 weeks to promote long-term neurogenesis and repair.

3.Combination Protocols:

In protocols combining EPO with hypothermia, EPO is typically given at the onset of cooling, with subsequent doses aligned to cooling sessions.

Combination with Hypothermia:

In the resource-rich setting, the gold standard management of moderate to severe HI Encephalopathy at term is TH (temperature decrease range of 2–5 C°) with the goal of keeping the core body temperature at 33.5 C for the first 72 hours of life, sometimes starting as early as the first six hours. In children with severe HIE, HT by alone is insufficient to prevent significant neurological problems or lower death. According to a meta-analysis by Mohamed A., 40 percent of newborns with HIE who received HT may die, have moderate to severe disabilities, or experience other significant impairments. They may also have other mortality rates. A 2017 review found that HT has been the most advanced recent development in the care of HIE at term, even if there are still many newborns for whom this therapy is futile.[37]

Thus, to avoid or treat brain injury in HIE, HT should be used in conjunction with other neuroactive medications, which is the purpose of modern studies on HIE.

Clinical trials were based on a study by **Dorothy E. et al** [38] which showed that EPO with hypothermia reduced cerebral palsy from neonatal hypoxia. Compared to hypothermia only,

study showed that treatment with EPO plus hypothermia yielded better 12-month motor outcomes of neonatal children with HIE in a recent Phase II randomised controlled trial.

Consequently, it can be suggested that both EPO and moderate hypothermia are efficient for the treatment for neonatal HIE. The neurological outcomes at 18-24 months and later are now needed as a result of two current clinical trials' In Phase I and II trials it has been established that EPO therapy mitigates outcomes of MRI observed brain injury; enhances neuro developmental results at 12-24 months of age, further large-scale phase III trials are still underway to establish longevity of the drug and its effects on the body. With EPO with hypothermia reduced cerebral palsy in neonatal hypoxia. In comparison to hypothermia alone, treatment with EPO plus hypothermia produced better 12-month motor results in neonatal children with HIE, according to a recent Phase II clinical trial. Therefore, it appears that EPO and moderate hypothermia together are an effective treatment for neonatal HIE. The results of two ongoing clinical trials on neurological outcomes at ages 18 to 24 months and older are now necessary.

Evidence from Studies:

Clinical Trials:

Several Phase I and II trials have shown that EPO therapy can reduce MRI-detected brain injury and improve neurodevelopmental outcomes at 12 to 24 months of age.

Large-scale Phase III trials are ongoing to confirm long-term efficacy and safety.

Animal Models: Cell culture studies have evidenced profound neuroprotection and enhanced functional outcome after HI injury.

Enhanced Neurological Frontiers Associated with Recombinant Erythropoietin in HIE [39,40]

recombinant erythropoietin has also indicated the effectiveness for improved neurological performances in neonates with hypoxic-ischemic encephalopathy. These advantages derive from its capacity to directly target both the early and delayed stages of neuronal damage, so providing improved lasting neuro-psychological and neurological performance.

Brain Lesion decrease (By MRI)

1.Imaging Studies: EPO-treated newborns have less severe MR images of the brain, primarily of hypoxic areas such as the cerebral white matter and basal ganglia and thalamus. Specific Findings: Less white matter injury, cystic evolution, and greater structural integrity of the brain

2. This indicates that there were no significant changes in motor function, such as walking and other upper extremity functions, from preintervention to one month, three months, six months, and one year after syncope. Lower Risk of Cerebral Palsy: EPO helps to reduce the number and severity of motor impairments, such as spasms and hypotonia.

Improved Coordination: Restore of the motor function, balance, and coordination in children taking with EPO.

3.Improved Learning and Cognitive Skills: Compared to untreated newborns, these babies have improved cognitive skill development, a higher IQ, and improved learning capacity when they start school. Memory and Attention: Both memory and attention are impacted in

HIE newborns, and EPO improves the brain plasticity that governs these functions.

4. Speech and Interaction The majority of children's language usage and interpersonal interaction style are essentially implied by their skills.

Verbal Skills: They improved their ability to read and write, expanded their vocabulary, and communicated more clearly. Increased self-skill in social integration, most likely due to better cognitive and affective functioning.

5.Enhancements in Behaviour

Decrease in Hyperactivity: Children's hyperactivity, aggression, and impulsivity all decrease when EPO is used. Improved Control of Emotions: Better regulation of energy or anger outbursts; reduced likelihood of depression; perhaps as a result of fewer cognitive and neuroanatomical alterations in specific brain regions.

6. Improved Visual and Auditory Skills

Vision: EPO therapy has been linked to better visual acuity and a lower prevalence of cortical visual impairment.

Hearing: By maintaining auditory processing, it may reduce the likelihood of hearing problems commonly associated with HIE.

7. Better White Matter and Gray Matter Development

Myelination: EPO promotes healthy myelination and white matter repair, two processes necessary for the brain's rapid transmission of signals. Development of the Cortical Layer: Maintains Gray matter integrity and growth, which is necessary for higher-order cognitive processes.

8. Long-Term Benefits for School-Aged Children Academic Achievement: Children who receive treatment are more likely to meet developmentally appropriate reading, writing, and

math benchmarks; a decreased need for special education services or assistive technology is an indication of independence;

9. Working in conjunction with Hypothermia to Enhance Neurological Results EPO enhances neuroprotection when therapy hypothermia is used, leading to even greater gains in motor and cognitive outcomes; both treatments improve general growth and lessen global brain damage in infants;

10. Benefits of Comparison Research shows that newborns treated with EPO outperform those treated with hypothermia alone in terms of cognitive, motor, and sensory outcomes. Safety Profile: EPO produces these benefits with few side effects when taken as directed.

Study Design and Methodology

Study Type: It is Prospective Comparative Study

Study Duration:12–18 months

Sample Size: 90 participants

Sample Size Calculation

The precise proportions are as follows: Inequality, two independent groups (unconditional); z-test (pooled); a priori analysis: Determine the necessary sample size.

Parameters of Input:

Allocation ratio (N2/N1): 1 Tail(s): Two

Odds ratio: 0.1457723

Proportion in control group (p2): 0.421

α error probability: 0.05

Power (1-).

• Results of the Output:

Group 1 and Group 2 sample sizes are 45 and 45, respectively. The total sample size is 90.

The actual α is 0.0469649 and the actual power is 0.9637553.

SAMPLE SIZE

Using G*Power ver 3.1.9.4 software for sample size calculation. The proportion of Brain injury on imaging for EPO is 42.1% and Control is 83.3%, this study requires a total sample size of 90(for each group 45, assuming equal group sizes), so to achieve a power of 80% for detecting a difference in Proportions: Exact - Proportions: Inequality, two independent group(unconditional) with 5% level of significance.

Statistical Analysis

- Data will be recorded in Microsoft Excel and analysed using SPSS software (Version 20).
- Results will be presented as Mean, SD, frequencies, percentages, and visual diagrams.
- **Tests Used:**

➢ **Continuous Variables will be used to interpret the** independent sample t-test for normally distributed data and Mann-Whitney U test for non-normally distributed data.

➢ **Categorical Variables:** Chi-square test or Fisher's exact test.

- **Significance Threshold:** $p < 0.05$ (two-tailed).

Methodology

Study Population:

The study will include all term and near-term newborns (≥36 completed weeks) with moderate to severe hypoxic-ischemic encephalopathy (HIE), as determined by the Sarnat and Sanat criteria.

Intervention:

- All neonates will receive intravenous recombinant human erythropoietin (rhEPO) after 6 hours of life.
- Total of 5 doses will be administered as follows:
    - **1st Dose:** Within 24 hours of life
    - **Subsequent Doses:** Day 2, Day 3, Day 5, Day 7
    - **Dosage:** 1000 IU/kg/dose

Monitoring:

- **RI (Resistive Index) via NSG:** Conducted on Day 1 to Day 3 of admission.
- **EEG Patterns:** Used to differentiate mild, moderate, and severe encephalopathy.
- **MRI Brain:** Performed by a radiologist prior to discharge to evaluate the grade of injury and prognosis.

Standard Care:

All newborns will receive standard intensive care during the study period.

Consent:

Informed parental consent will be obtained at the start of the study.

Inclusion Criteria:

1. Neonates ≥36 weeks gestation.
2. Birth weight >1.8 kg with moderate to severe encephalopathy.
3. Admission to NICU after 6 hours of life.

Exclusion Criteria:

1. Lethal congenital malformations or genetic conditions affecting neurodevelopment.
2. Evidence of bleeding diathesis.
3. Haematocrit >65%.

Study Outcomes:

1. Changes in EEG patterns indicative of moderate/severe encephalopathy.
2. Alterations in abnormal RI patterns on NSG.
3. MRI brain findings prior to discharge.

Results

Table 1: Distribution of Encephalopathy in EPO and Control Groups

Sl.no	ENCEPHALOPATHY	EPO group n=46	Control Group n=46
1	Moderate	22(47.8%)	18(39.1%)
2	Severe	24(52.2%)	28(60.9%)
3	Total	46(100%)	46(100%)

This table presents the distribution of encephalopathy in neonates who received human recombinant erythropoietin (EPO) versus the control group. In the EPO group, 47.8% (n=22) had moderate encephalopathy, while 52.2% (n=24) had severe encephalopathy. In comparison, the control group showed 39.1% (n=18) with moderate encephalopathy.

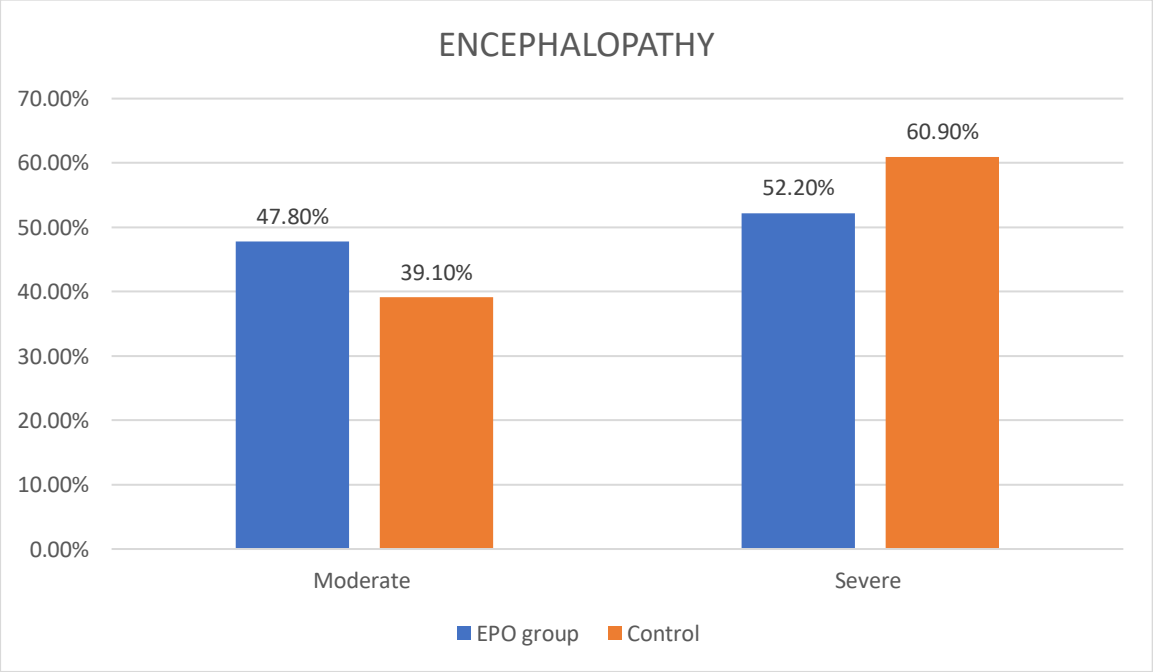


Figure 11: Distribution of Encephalopathy in EPO and Control Group

Table 2: Distribution of Day of life of newborn with Received EPO group and control group.

Sl.no	DAY OF LIFE	EPO group n=46	Control Group n=46
1	at 6 hours of life	2(4.3%)	0
2	1 day of life (6 to 24hrs)	43(93.5%)	43(93.5%)
3	2 days of life (>24hrs)	1(2.2%)	3(6.5%)

This table presents the distribution of the day of life in neonates who received human recombinant erythropoietin (EPO) versus the control group. Findings show that in both the EPO and control groups, 93.5% (n=43) had a duration of life of 1 day. The data is visually represented in the accompanying bar diagram.

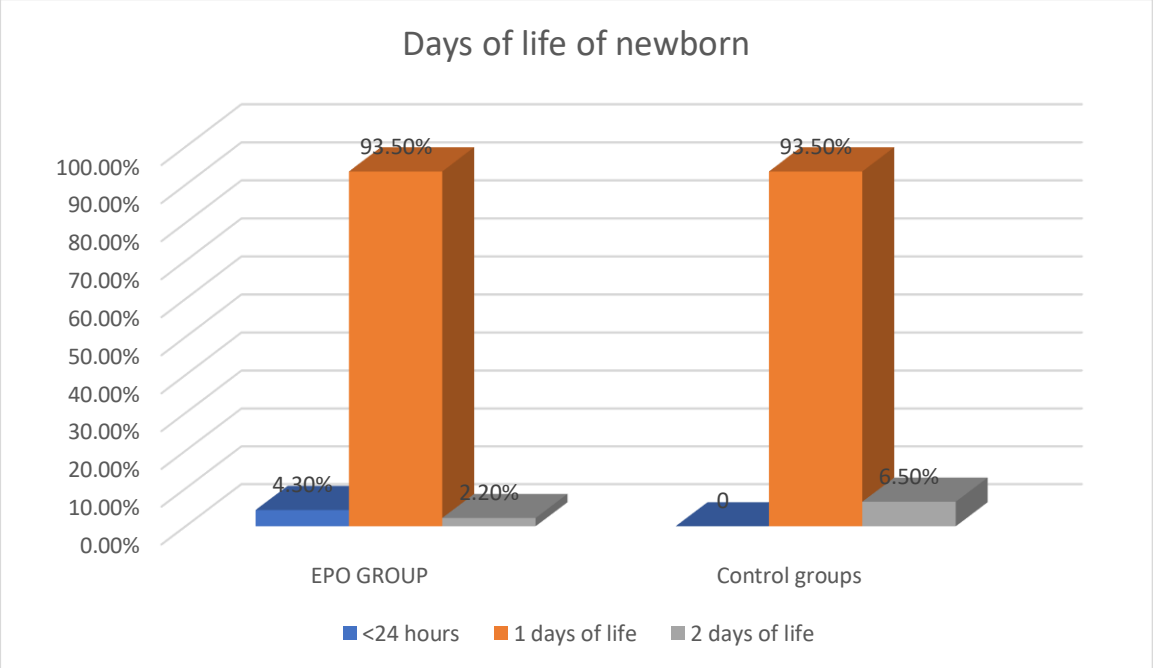


FIGURE 12: Distribution of Day of life of newborn with EPO group and control group

Table 3: Distribution of Gestational week with Human Recombinant Erythropoietin therapy (EPO)among cases and control group

Sl.no	GESTATIONAL WEEK	EPO group n=46	Control Group n=46
1	36 to <37 weeks	4 (8.7%)	3(6.5%)
2	>37 weeks	42(91.3%)	43(93.5%)
3	Total	46(100%)	46(100%)

This table presents the distribution of gestational age among the EPO and control groups. In the EPO group, 91.3% (n=42) had a gestational age of more than 37 weeks, while 8.7% (n=4) had a gestational age of less than 37 weeks. The data is visually represented in the accompanying pie chart.

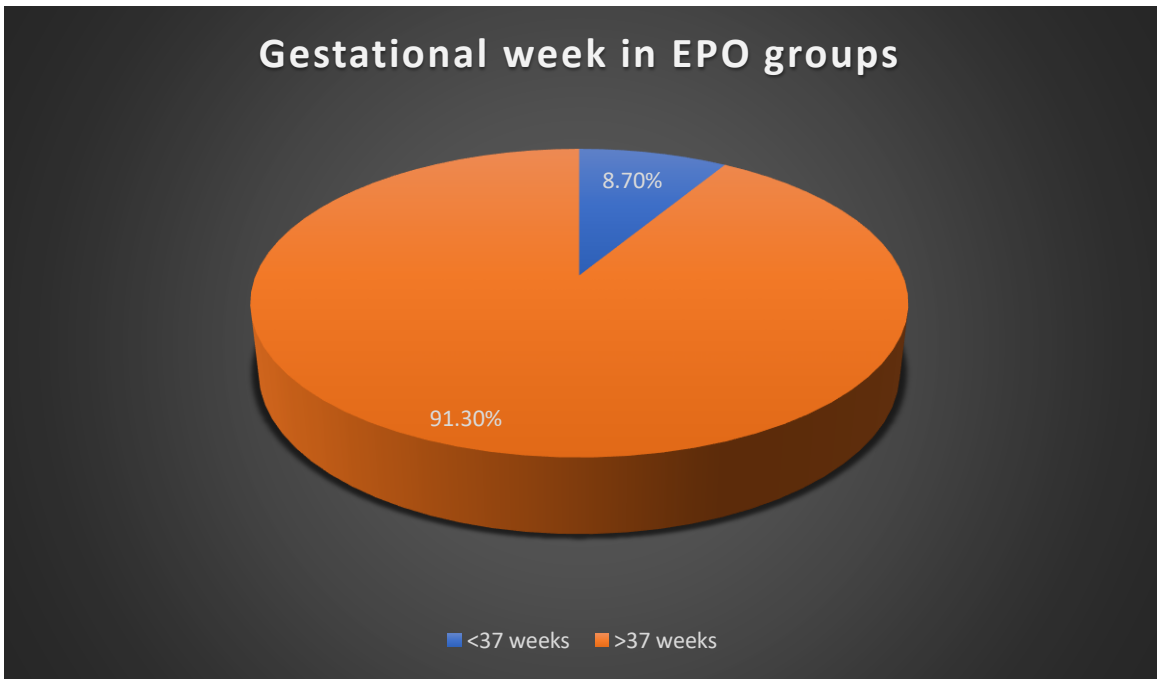


FIGURE 13: **Distribution of gestational week with Human Recombinant Erythropoietin therapy (EPO)among cases and control group**

Table 4: **Distribution of GRBS with Human Recombinant Erythropoietin therapy (EPO)among cases and control group**

Sl.no	GRBS AT ADMISSION	EPO group n=46	Control Group n=46
1	Hyperglycemia	3(6.5%)	4(8.7%)
2	Normal	43(93.5%)	42(91.3%)
3	Total	46(100%)	46(100%)

The distribution of GRBS at admission among EPO and control groups is illustrated in the table and represented in a pie chart. In the EPO group, 93.5% (43) of patients had normal GRBS at admission, while only 6.5% (3) presented with hyperglycemia.

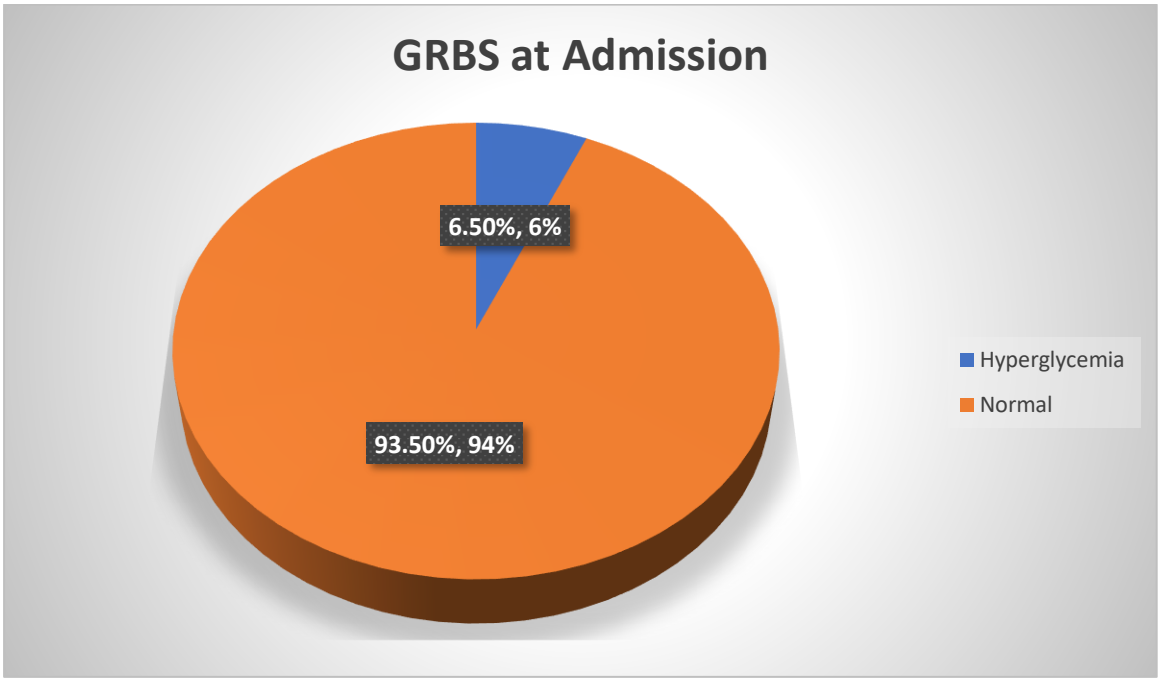


FIGURE 14: Distribution of GRBS at admission with EPO group and control group

Table 5: Distribution of SEX with EPO group and control group.

Sl.no	SEX	EPO group n=46	Control Group n=46
1	Female	16(34.8%)	10(21.7%)
2	Male	30(65.2%)	36(79.3%)
3	Total	46(100%)	46(100%)

The table presents the distribution of sex among EPO and control groups, as depicted in the bar diagram. In the EPO group, 65.2% (n=30) were males and 34.8% (n=16) were females. Similarly, in the control group, 79.3% (n=36) were males, while 21.7% (n=10) were females.

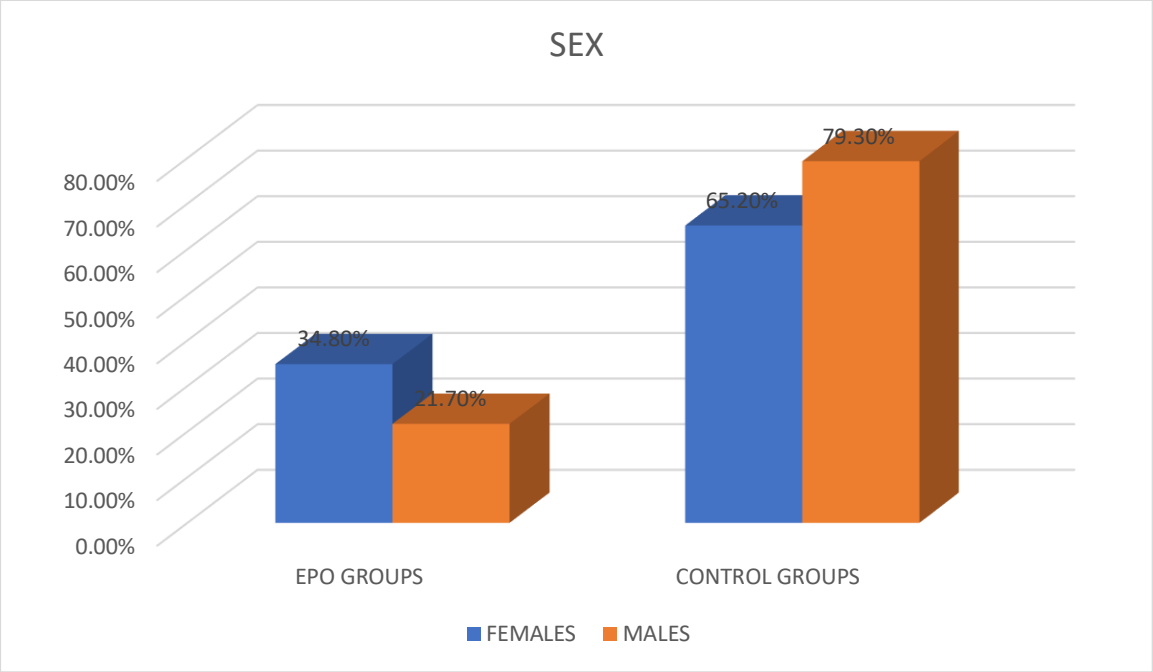


Figure 15: Distribution of SEX with EPO group and control group

Table 6: Distribution of Birth Weight in Human Recombinant Erythropoietin (EPO) and Control Groups.

Sl.no	BIRTH WEIGHT	EPO group n=46	Control Group n=46
1	1.8-2.5kgs	14(30.4%)	4(8.7%)
2	2.5-3.5kgs	31(67.4%)	38(82.6%)
3	>3.5kgs	1(2.2%)	4(8.7%)

The table presents the distribution of birth weight among the EPO and control groups, as illustrated in the bar diagram. The majority of newborns in the EPO group (67.4%, n=31) had a birth weight between 2.5–3.5 kg, similar to the control group (82.6%, n=38). Additionally, 30.4% (n=14) of newborns in the EPO group had a birth weight of less than 2.5 kg.

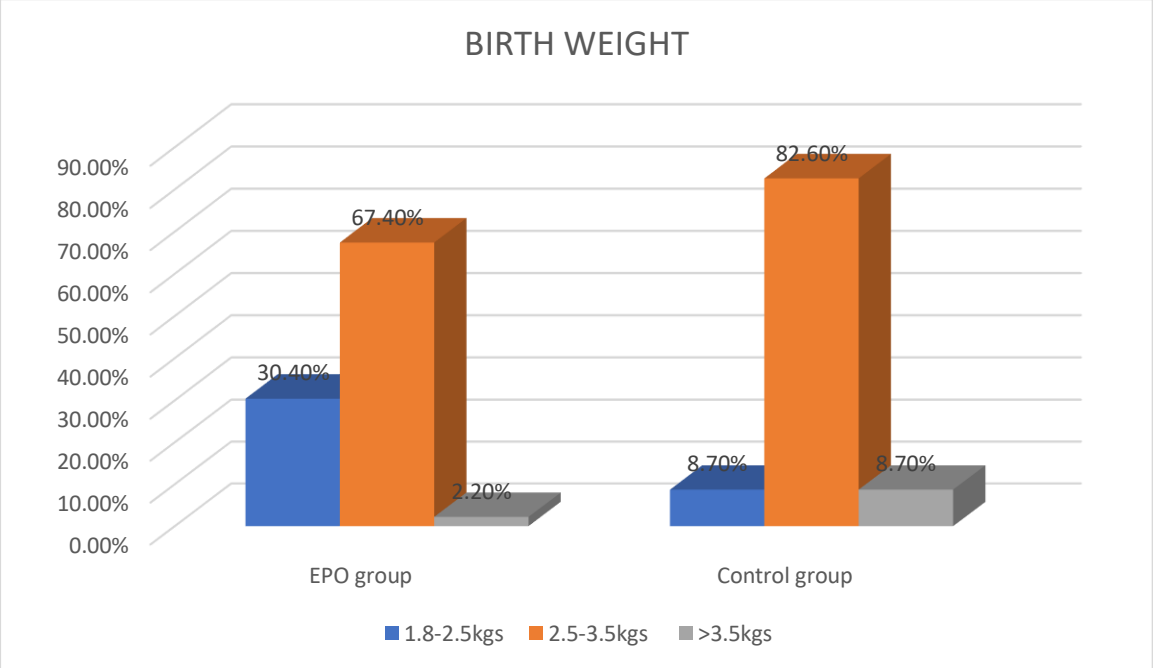


FIGURE 16: Distribution of birth weight with HUMAN RECOMBINANT ERYTHROPOIETIN (EPO) group and control group

Table 7: **Distribution of Parity in Human Recombinant Erythropoietin (EPO) and Control Groups**

Sl.no	PARITY	EPO group n=46	Control Group n=46
1	Multigravida	28(60.9%)	17(37%
2	Primigravida	18(39.1%)	29(67%)
3	Total	46(100%)	46(100%)

The table presents the distribution of maternal parity in the EPO and control groups, as illustrated in the pie chart. In the EPO group, 60.9% (n=28) of mothers were multigravida, while 39.1% (n=18) were primigravida. In comparison, 37% (n=17) of mothers in the control group were primigravida.

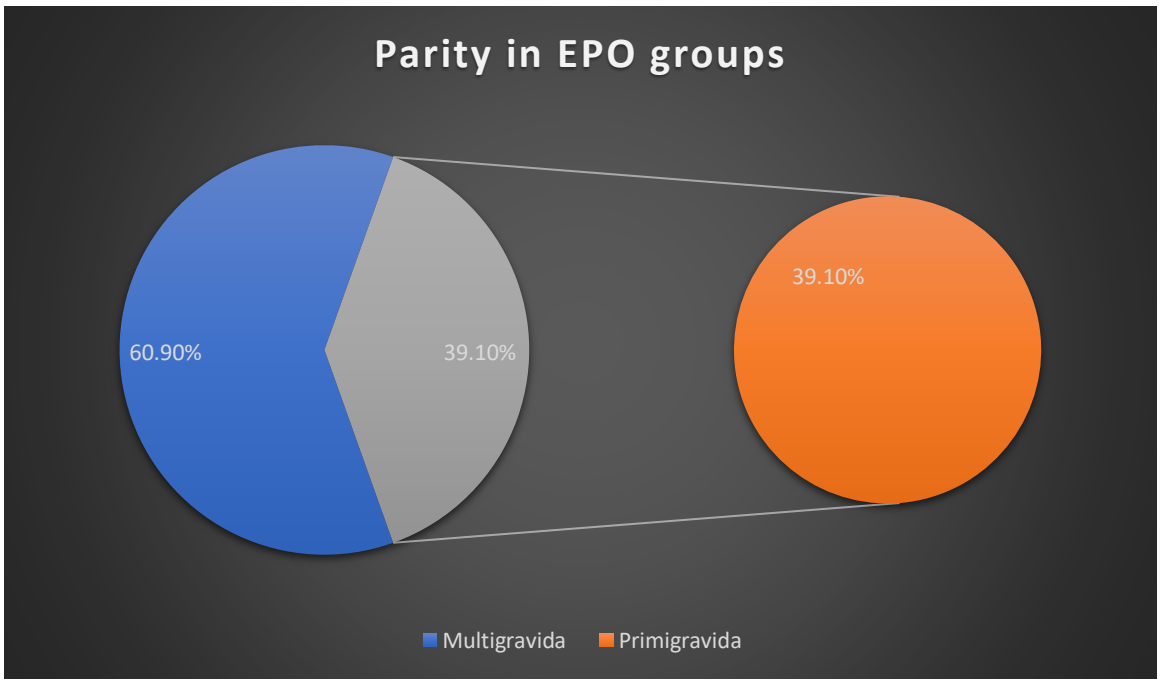


Figure 17: Distribution of Parity with HUMAN RECOMBINANT ERYTHROPOIETIN (EPO) and control group

Table 8: Distribution of maternal age with HUMAN RECOMBINANT ERYTHROPOIETIN (EPO) and control group

Sl.no	MATERNAL AGE	EPO group n=46	Control Group n=46
1	<19 years	2(4.3%	1(2.2%)
2	20-35years	44(95.7%)	44(95.7%)
3	>35 YEARS	0.	1(2.2%)

The table presents the distribution of maternal age in the EPO and control groups, as illustrated in the bar diagram. In the EPO group, 95.7% (n=44) of mothers were within the 20–35 years age range, a finding that aligns with the control group.

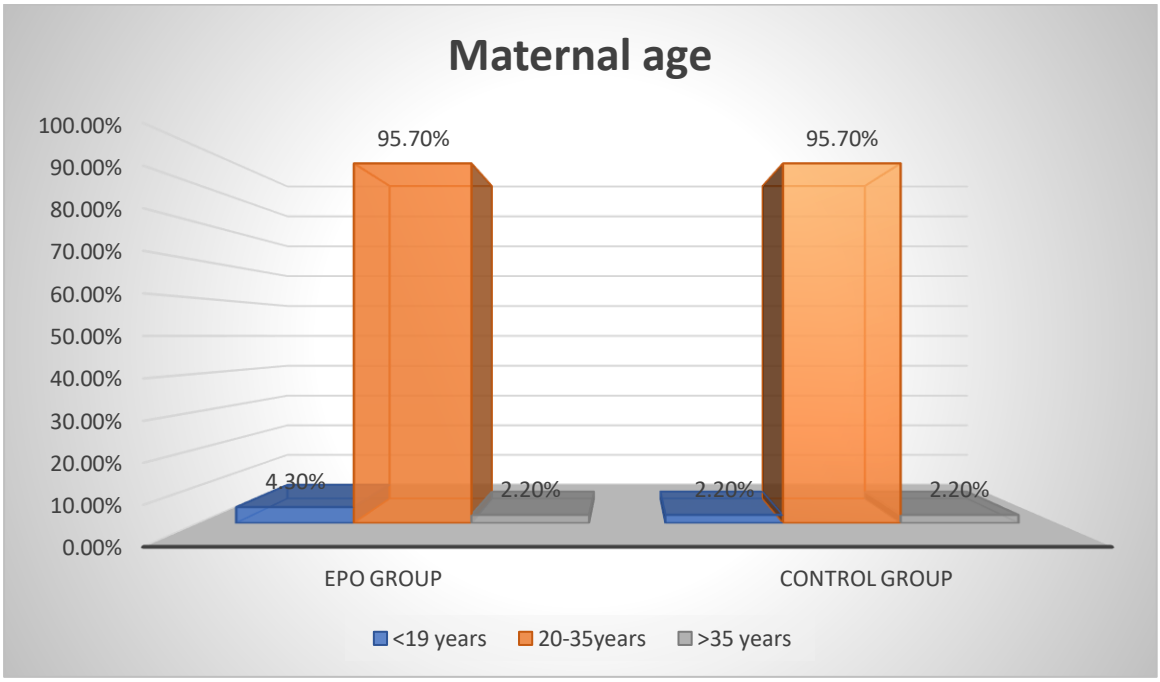


Figure 18: Distribution of maternal age with HUMAN RECOMBINANT ERYTHROPOIETIN (EPO) and control group

Table 9: **Distribution of Pregnancy Complications in Human Recombinant Erythropoietin (EPO) and Control Groups**

Sl.no	PREGANCY COMPLICATIONS	EPO group n=46	Control Group n=46
1	GESTATIONAL DIABETES	2(4.3%)	7(15.2%)
2	GESTATIONAL HYPERTENSION	12(26.1%)	12(26.1%)
3	OTHERS COMPLICATION	15(32.6%)	11(23.9%)
4	Nil	17(37.0%)	16(34.8%)

The table presents the distribution of pregnancy complications among the EPO and control groups, as illustrated in the pie diagram. In the EPO group, 26.1% (n=12) of mothers had gestational hypertension, while 37% (n=17) had no complications during pregnancy

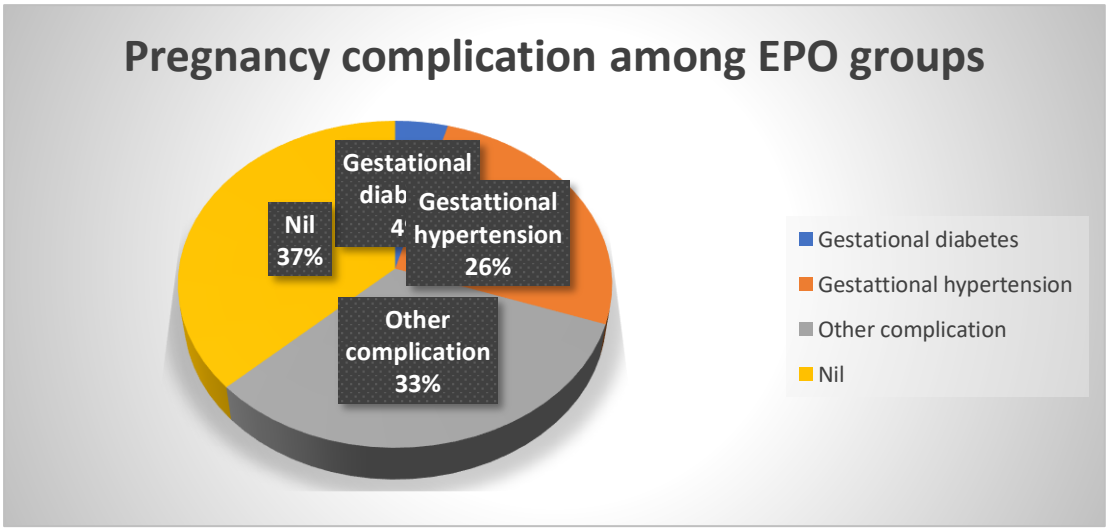


Figure 19: **Distribution of Pregnancy Complications in Human Recombinant Erythropoietin (EPO) and Control Groups**

Table 10: Distribution of Mode of Delivery in Human Recombinant Erythropoietin (EPO) and Control Groups

Sl.no	MODE OF DELIVERY	EPO group n=46	Control Group n=46
1	LSCS	18(39.1%)	18(39.1%)
2	NVD	28(60.9%)	28(60.9%)
3	Total	46(100%)	46(100%)

The table presents the distribution of mode of delivery in the EPO and control groups. In both groups, 60.9% (n=28) of deliveries were normal vaginal deliveries, while 39.1% (n=18) were via lower segment caesarean section (LSCS).

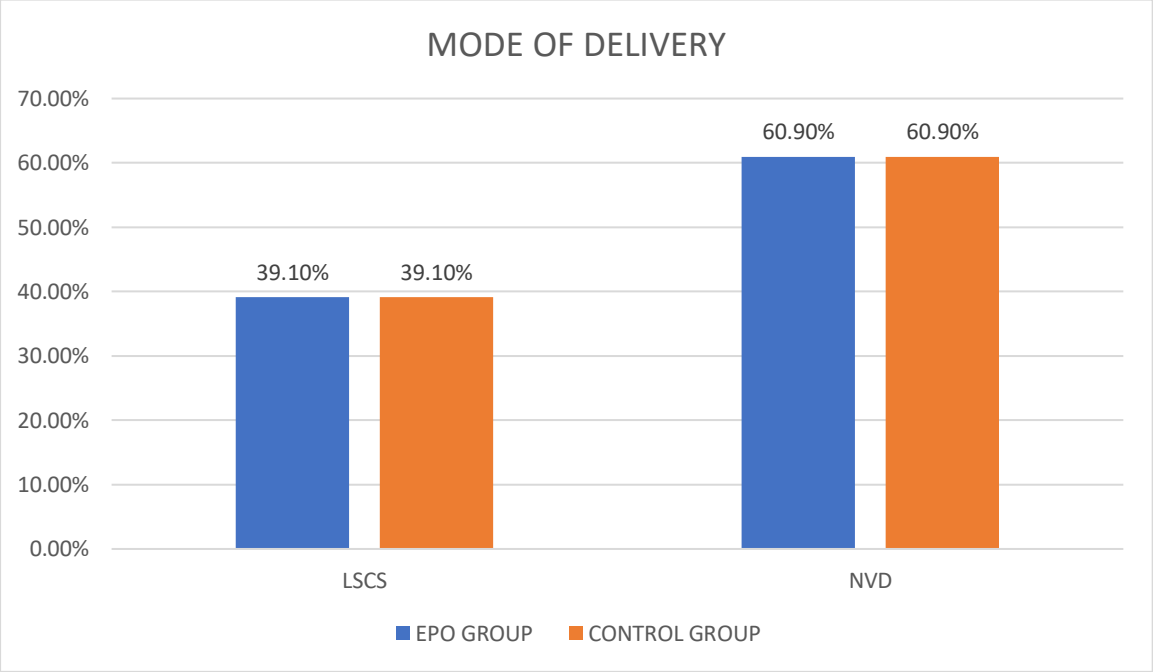


Figure 20: **Distribution of Mode of Delivery in Human Recombinant Erythropoietin (EPO) and Control Groups.**

Table 11: Association Between Delivery complication in Human Recombinant Erythropoietin (EPO) and Control Groups

Sl.no	Delivery Complication	EPO group n (%)	Control Group n (%)	P value
1	ABNORMAL FETAL HEART RATE	8(17.4%)	6(13.0%)	0.726 1.31 3
2	MECONIUM OR STAINED AMNIOTIC FLUID	28(56.5%)	27(58.7%)	
3	OTHER complication	1(2.2%)	3(6.5%)	
	Nil	11(23.9%	10(21.86%)	

The table shows the distribution of delivery complications in the EPO group. In this group, 56.5% (n=28) of babies had meconium-stained amniotic fluid, while 17.4% (n=8) exhibited abnormal fetal heart sounds. After applying the chi-square test, the p-value was found to be non-significant (>0.05).

Table 12: Distribution of peripartum abnormalities with HUMAN RECOMBINANT ERYTHROPOIETIN (EPO) and control group

Sl no	Peripartum Abnormalities	EPO group n=46	Control Group n=46
1	Present	14(29.4%)	7(16.2%)
2	Absent	32(69.6%)	38(84.8%)
3	Total	46(100%)	46(100%)

The table shows the distribution of delivery complications in the EPO and control groups. In the EPO group, 29.4% (n=14) of cases involved peripartum abnormalities such as uterine prolapse, abruptio placenta, and cord prolapse. In contrast, only 16.2% (n=7) of cases in the control group experienced similar complications.

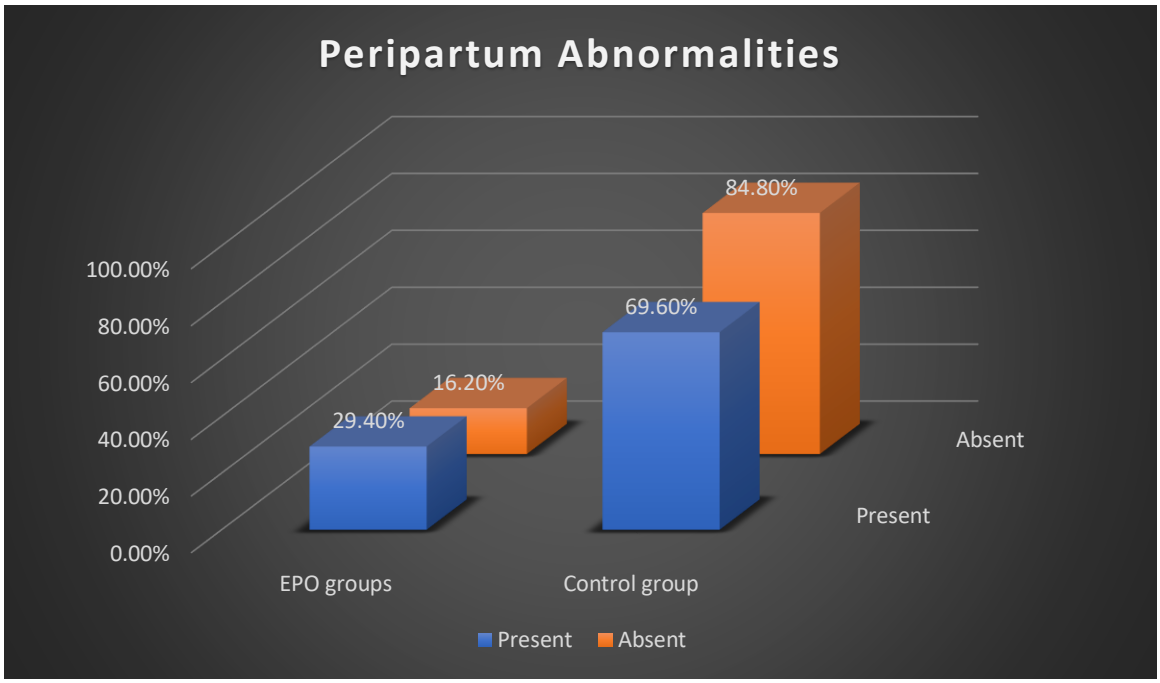


Figure 21: Peripartum abnormalities with HUMAN RECOMBINANT ERYTHROPOIETIN (EPO) and control group

Table 13: Distribution of Birth order with HUMAN RECOMBINANT ERYTHROPOIETIN (EPO) and control group

Sl.no	BIRTH ORDER	EPO group n=46	Control Group n=46
1	First	18(39.1%)	29(63%)
2	Second	17(37%)	6(13%)
3	>3rd birth order	11(23.9%)	11(23.9%)

The table presents the distribution of delivery complications in the EPO and control groups based on birth order. In the EPO group, the majority (39.1%, n=18) were first-time mothers, followed by 37% (n=17) who had their second child. In comparison, the control group had 63.5% (n=29) first-time mothers.

Table 14: Association between of Mode of Resuscitation with HUMAN RECOMBINANT ERYTHROPOIETIN (EPO) and control group

Sl.no	Mode of Resuscitation	EPO group n=46	Control Group n=46	P value
1	After STIMULATION	13(28.3%)	9(19.6%)	P value - 0.263 X^2= 2.663 Df-2
2	INTUBATION	4(8.7%)	9(19.6%)	
3	POSITIVE PRESSURE VENTILATION	29(63.0%)	28(60.9%)	

The table shows the distribution of delivery complications in the EPO group for newborns with hypoxic-ischemic encephalopathy. Among these infants, 63% (n=29) were given positive pressure ventilation, while 28.3% (n=13) received resuscitation through stimulation. The difference in resuscitation methods was not statistically significant, with a p-value greater than 0.05.

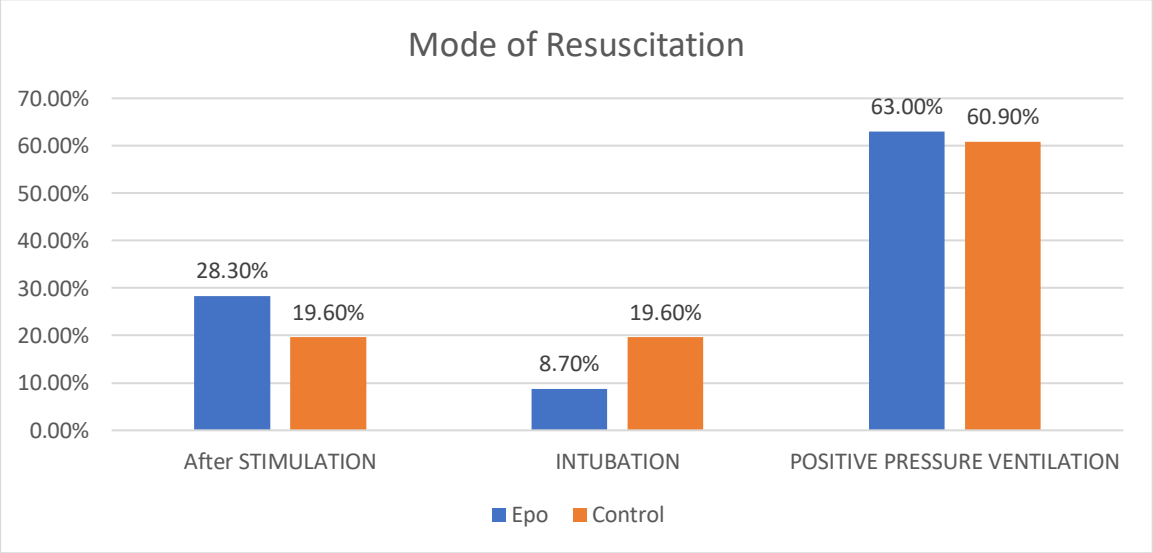


Figure 22: **Association between of Mode of Resuscitation with HUMAN RECOMBINANT ERYTHROPOIETIN (EPO) and control group.**

Table 15: **Distribution of NICU Admission Duration Among Infants with Hypoxic-Ischemic Encephalopathy in Human Recombinant Erythropoietin (EPO) and Control Groups**

Sl no	Nicu Admissions in Hrs of Life	EPO group n=46	Control Group n=46
1	LESS THAN OR EQUAL TO 6 HRS	1(2.2%)	1(2.2%)
2	MORE THAN 6 HRS AND LESS THAN 1 DAY	45(97.8%)	44(93.5%)
3	More than 1 day	0	2(4.3%)

The table shows the distribution of NICU admission duration for neonates with hypoxic-ischemic encephalopathy in the EPO and control groups. In the EPO group, 97.8% (n=45) of babies had an admission duration of more than 6 hours but less than 1 day, while only 2.2% (n=1) were admitted for less than 6 hours. In comparison, the control group had 93.5% of neonates with an admission duration greater than 6 hours, as shown in the bar diagram.

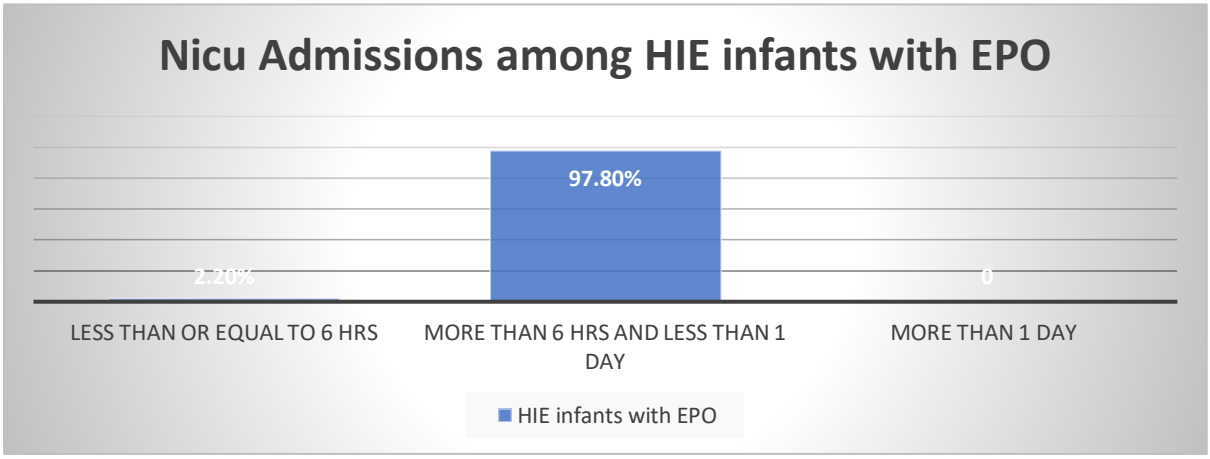


Figure 23: Distribution of duration of NICU Admission among Hypo-Ischemic encephalopathy baby with HUMAN RECOMBINANT ERYTHROPOIETIN (EPO) group and control group

Table 16: **Distribution of Primary Respiratory Support Among Neonates with Hypoxic-Ischemic Encephalopathy in Human Recombinant Erythropoietin (EPO) and Control Groups**

Sl.no		EPO group n (%)	Control Group n (%)	P value
Primary Respiratory Support	HFNC	32(69.64%)	26(56.5%)	P value – 0.17 X^2= 4.97 Df-3
	HOOD OXYGEN	5(10.9%)	3(6.5%)	
	NASAL PRONGS	2(4.3%)	1(2.2%)	
	SIMV	7(15.2%)	16(34.8%)	
DURATION OF REPIRATORY SUPPORT	less than 48hrs	7(15.2%)	3(6.5%)	P value - **<0.001** X^2= 47.57 Df-2
	3 days to 7 days	36(78.3%)	28(59.5%)	
	more than 7 days	3(6.5%)	15(32.6%)	

The table presents the distribution of primary respiratory support for neonates with hypoxic-ischemic encephalopathy in the EPO and control groups. In the EPO group, 69.64% of babies received high-flow nasal cannula (HFNC), followed by 15.2% requiring synchronized intermittent mandatory ventilation (SIMV). In the control group, 56.5% of neonates received HFNC. However, the difference in the type of respiratory support was not found to be statistically significant (p-value > 0.05).

Regarding the duration of respiratory support, we found that 78.3% (n=36) of newborns in the EPO group required 3–7 days of respiratory support, whereas 59.5% (n=28) of the control group required 3-7 days. Whereas (6.5% vs 32.6%) newborns needed more than 7 days in the EPO group and control group respectively. This difference in duration of respiratory support was found to be statistically significant.

Table 17: **Association Between Neonates Developing Convulsions in Hypoxic-Ischemic Encephalopathy with Human Recombinant Erythropoietin (EPO) and Control Groups**

Variables		EPO group n (%)	Control Group n (%)	P value
CONVULSION DURING TREATMENT	YES	28(60.9%)	36(80.5%)	P value – 0.25 X^2= 0.465 Df-1
	NO	10(21.7%)	9(19.7%)	
ANTI CONVULSANTS LOADED DURG CONVULSIONS	YES	28(60.9%)	36(80.5%)	P value – 0.25 X^2= 4.65 Df-1
	NO	10(21.7%)	9(19.7%)	
FIO2 REQUIREMENT	less than 30%	26(56.5%)	3(6.5%)	**Pvalue–<0.001** X^2= 32.7 Df-2
	30 to 60%	18(39.1%)	31(67.4%)	
	more than 60%	2(4.3%)	12(26.1%)	
DURATION OF NICU STAY	3 to 7 days	29(63%)	21(45.5%)	**P value –< 0.05** X^2= 2.8 Df-1
	more than 7 days	17(37%)	25(54.5%)	

This table presents the occurrence of neonatal convulsions among infants with Hypoxic-ischemic encephalopathy receiving Human Recombinant Erythropoietin (EPO) treatment, compared to the control group. The results show no significant association between the

development of convulsions and EPO treatment (p-value > 0.05). However, a significant difference was observed in the oxygen requirements between the EPO and control groups, with this difference found to be statistically significant.

Table 18: Association Between Laboratory Values at admission in Neonates with Hypoxic-Ischemic Encephalopathy in Human Recombinant Erythropoietin (EPO) and Control Groups

Variables	EPO group Mean (SD)	Control Group Mean (SD)	P value
Hemoglobin level at admission	16.5(2.18)	17.17(2.9)	**0.005**
PCV (HEMATOCRIT AT ADMISSION)	49.8(6.45)	51.5(59)	**<0.0001**
TOTAL COUNT AT ADMISSION	23628.04(25268)	26106.7(28547)	0.4162

This table presents the mean (SD) values of haemoglobin level, packed cell volume, and total white blood cell count at admission. The values were 16.5 (2.18) for haemoglobin, 49.8 (6.45) for packed cell volume, and 23,628 (25,268) for the total white blood cell count. A significant difference was found in the haemoglobin level and packed cell volume, but no significant association was observed with the total white blood cell count at admission.

Table 19: **Association Between Laboratory Values at 48th hour in Neonates with Hypoxic-Ischemic Encephalopathy in Human Recombinant Erythropoietin (EPO) and Control Groups**

Variables	EPO group Mean (SD)	Control Group Mean (SD)	P value
Hemoglobin level at admission	16.81(1.61)	15.90(2.45)	0.0637
PCV (HEMATOCRIT AT ADMISSION)	49.43(5.42)	47.62(7.13)	0.2232

These values were found to be statistically insignificant between the EPO and Control groups.

Table 20: **Association between blood gas parameters (arterial blood gas (ABG)) among Hypo-Ischemic encephalopathy baby with HUMAN RECOMBINANT ERYTHROPOIETIN (EPO) Treatment with control group**

Sl. no	Variables	EPO group Mean (SD)	Control Group Mean (SD)	P value
1	PH ADMISSION	7.3(0.11)	7.2(0.12)	P value –0.561
2	PCO2 AT ADMISSION	23.7(6.31)	23.3(6.2)	P value –0.9066
3	PO2 AT ADMISSION	123.93(48.99)	141.6(61.5)	P value –0.130
4	HCO3 AT ADMISSION	12.3(3.60)	11.39(3.4)	P value –0.703

This table presents the blood gas parameters (arterial blood gas - ABG) at admission, with the mean (SD) values for pH, pO2, pCO2, and HCO3 as follows: 7.3 (0.11) for pH, 23.7 (6.31) for

pO2, 123.93 (48.99) for pCO2, and 12.3 (3.60) for HCO3. These values were found to not be statistically significant.

Table 21: Association Between Biochemical Parameters in Neonates with Hypoxic-Ischemic Encephalopathy in Human Recombinant Erythropoietin (EPO) and Control Groups

Sl.no	Biochemical parameters	EPO group Mean (SD)	Control Group Mean (SD)	P value
1	SODIUM AT 48HRS	139.66(6.00)	139.3(6.1)	P value -0.9122
2	POTASSIUM AT 48HRS	4.5(0.84)	4.45(0.92)	P value -0.5442
3	CALCIUM AT 48HRS	7.5(1.2)	7.5(1.2)	P value -0.999

This table presents the biochemical parameters in neonates affected by hypoxic-ischemic encephalopathy (HIE). The mean and standard deviation values at the 48th hour of admission are as follows: sodium level 139.66 (6.00), potassium level 7.5 (1.2), and calcium level 7.5 (1.2). These values are comparable to those in the control group and were found to be statistically insignificant.

Table 22: **Distribution of Cerebral Function Monitoring (CFM), aEEG (Electroencephalogram) Findings after 48hrs to 72hrs after admission Among Neonates with Hypoxic-Ischemic Encephalopathy in Human Recombinant Erythropoietin (EPO) and Control Groups.**

Sl.no	**Cerebral Function Monitoring (CFM) findings**	EPO group n (%) n=46	Control Group n (%) n=46	P value
1	BURST SUPRESSION	3(6.5%)	10(21.7%)	**P value-<0.001**
2	CONTINUOUS	22(47.8%)	4(8.7%)	**P value-<0.001**
3	CONTINUOUS LOW VOLTAGE	2(4.3%)	5(10.9%)	**P value-<0.001**
4	DISCONTINUOUS	14(30.4%)	18(39.1%)	P value-0.094
5	FLAT TRACE	4(8.7%)	6(13.0%)	**P value-<0.001**
6	STATUS EPILEPTICUS	1(2.2%)	3(6.5%)	**P value-<0.001**

This table presents the CFM findings among neonates with Hypoxic-Ischemic Encephalopathy in the Human Recombinant Erythropoietin (EPO) and control groups. It was found that the majority of neonates in the EPO group exhibited continuous CFM findings, while 39.1% (n=18) in the control group had discontinuous CFM findings. This difference was statistically significant, as shown in the bar diagram.

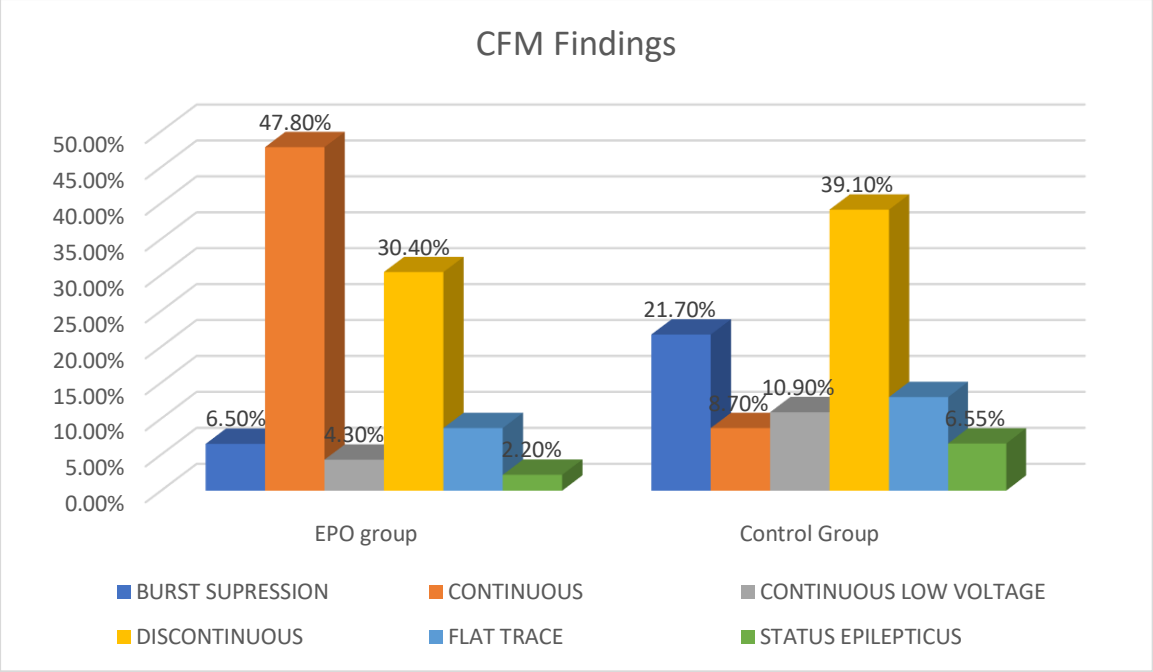


Figure 24: Distribution of CFM findings among Hypo ischemic encephalopathy neonates with HUMAN RECOMBINANT ERYTHROPOIETIN (EPO) group and control group

Table 23: Association between NSG findings with EPO and control group

Sl.no	NSG Findings	EPO group n=46	Control Group n=46	P value
1	ABNORMAL RI	7(15.2%)	26(56.5%)	**P value-<0.001**
2	NORMAL RI	39(84.8%)	20(43.5%)	

X^2= 17.06, Df-1

This table presents the NSG (Neurosonogram) findings among neonates affected by Hypoxic-Ischemic Encephalopathy (HIE) in both the Human Recombinant Erythropoietin (EPO) and control groups. It was found that 84.8% of neonates in the EPO group had a normal Resistive Index (RI), compared to 43.5% (n=20) in the control group. This difference was statistically significant, as depicted in the bar diagram.

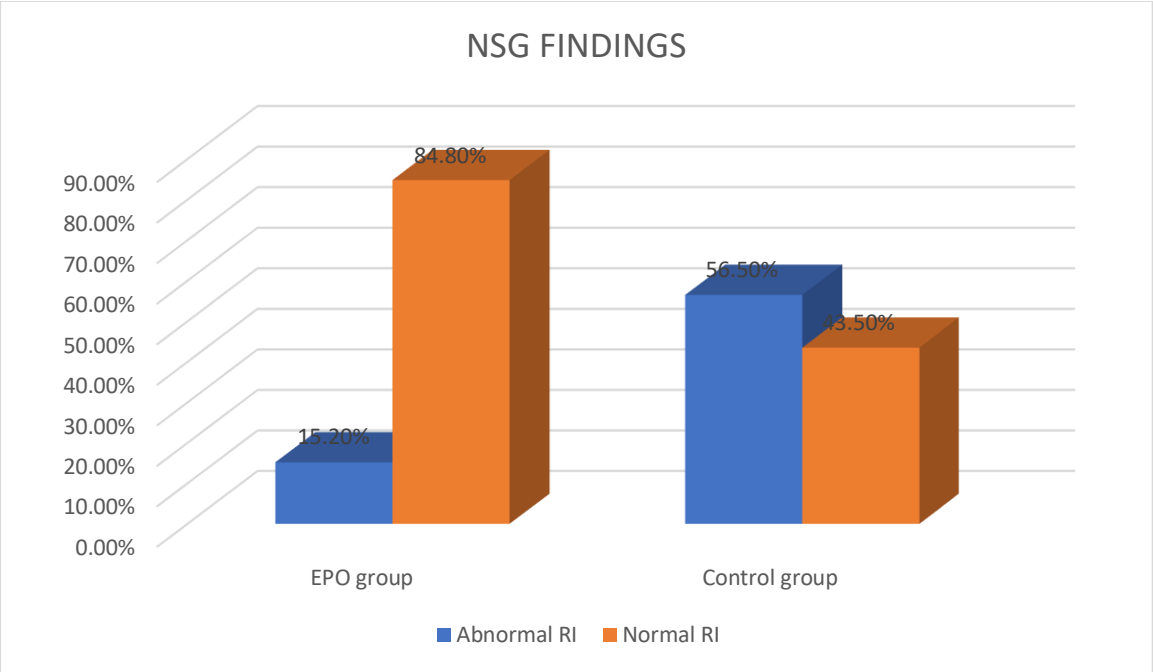


Figure 25: Association between NSG findings with EPO and control groups

Table 24: Association between MRI findings with EPO and control groups

Sl.no	MRI FINDINGS	EPO group n=25	Control Group n=25	P value
1	REGIONAL SPECIFIC HIE	18(39.1%)	9(19.5%)	**P value<0.005**
2	SEVERE HIE	4(8.6%)	15(32.6%)	
3	Normal study	3(6.5%)	1(2.1%)	

X^2= 10.37Df-2

This table presents the MRI findings among neonates affected by Hypoxic-Ischemic Encephalopathy (HIE) in both the Human Recombinant Erythropoietin (EPO) and control groups. It was found that 39.1% (n=18) of the neonates in the EPO group had regional-specific HIE, while only 8.6% (n=4) had severe HIE. In comparison, the control group had 32.6%(n-15) with severe HIE. This difference was found to be statistically significant.

Table 25: Association between development of Neonatal seizures with EPO and control groups

Sl.no	NEONATAL SEIZURE	EPO group n-27	Control Group n-35	P value
1	ABNORMAL RI	5(18.5%)	23(65.7%)	**P value-<0.001**
2	NORMAL RI	22(81.4%)	12(34.2%)	

X^2= 13.71, Df-2

This table presents the NSG (Neurosonogram) findings among neonates affected by Hypoxic-Ischemic Encephalopathy (HIE) in both the Human Recombinant Erythropoietin (EPO) and control groups. Among the 46 infants in the EPO group, 27 developed neonatal seizures. Of these, 81.4% (n=22) had a normal Resistive Index (RI), while 18.5% (n=5) had an abnormal RI. In comparison, 65.7% (n=23) of infants in the control group had an abnormal RI. This difference was found to be statistically significant.

Table 26: Association between development of ANTICONVULSANTS REQUIRED TO CONTROL SEIZURE among HIE affected baby in both case (EPO) groups and control groups

Variables	NSG Findings	EPO group	Control Group	P value
NUMBER OF ANTICONVULSANTS REQUIRED TO CONTROL SEIZURE -1	ABNORMAL RI	3(6.5%)	12(26.1%)	**P value – < 0.01** X^2= 10.26 Df-1
	NORMAL RI	23(50%)	10(21.7%)	
NUMBER OF ANTICONVULSANTS REQUIRED TO CONTROL SEIZURES >1	ABNORMAL RI	2(4.3%)	16(34.8%)	**P value – < 0.07** X^2= 4.12 Df-1
	NORMAL RI	3(6.5%)	3(6.5%)	
ANTICONVULSANTS FOR NEONATES' SEIZURE AT DISCHARGE	ABNORMAL RI	4(8.7%)	18(39.1%)	**P value – < 0.01** X^2= 12.45 Df-1
	NORMAL RI	22(47.8%)	11(23.9%)	
ON INOTROPES	ABNORMAL RI	7(15.2%)	27(58.5%)	**P value – < 0.01** X^2= 18.45 Df-1
	NORMAL RI	38(82.6%)	19(41.3%)	

This table shows the association between the development of anticonvulsants required to control seizures among neonates with Hypoxic-Ischemic Encephalopathy (HIE) in both the

Human Recombinant Erythropoietin (EPO) and control groups. It was found that the requirement for anticonvulsants to manage seizures, as well as the use of inotropes, were statistically significant. The p-value was found to be <0.001 after applying Fisher's exact test.

Table 27: Distribution of Final outcome among Hypo-Ischemic encephalopathy baby with HUMAN RECOMBINANT ERYTHROPOIETIN (EPO) Treatment with control group.

Sl no	Final outcome	EPO group n=46	Control Group n=46	P value
1	Discharged	45(97.8%)	41 (89.1%)	<0.005
2	Death	1(2.17%)	5 (10.8%)	
4	Total	46(100%)	46(100%)	

X^2= 3.2Df-2

This table presents the final outcome among both the groups and found that 97.8% discharged among cases and 89.1% among control. ONE Death happened in cases but 10.8%(n-5) death happened among control groups and this value is found to be significant

Discussion

The study titled Role of Human Recombinant Erythropoietin in Moderate to Severe Hypoxic Ischemic Encephalopathy in Neonates A Prospective Comparative Study Done on A 92 Study Participants With a objective to assess the safety and feasibility of rhEPO in asphyxiated neonates with moderate to severe encephalopathy and to know and to correlate the effect of EPO on aEEG, RI (resistive index) in Neuro-sonogram (NSG), MRI brain in asphyxiated neonates with moderate to severe encephalopathy.

Hypoxic-ischemic encephalopathy (HIE) functions as major contributor to neonatal mortality and morbidity because it produces long-term disabilities that include cerebral palsy with epilepsy alongside cognitive deficits. Hypothermia plays a key role as the standard therapy for HIE patients who have moderate-to-severe conditions because studies demonstrate better survival chances and lower risk of neurological disabilities. The benefits of therapeutic hypothermia exist but the treatment fails to prevent all detrimental post-treatment results. Additional interest in human recombinant erythropoietin (EPO) treatment as an adjunct therapy has risen because this agent shows potential to protect brain cells by decreasing secondary damage pathways involving inflammation and oxidative stress and apoptosis.

In our present study we find that the EPO group had 47.8% neonates with moderate encephalopathy while the control group only had 52.2% affected neonates. The EPO treatment group consisted of neonates whose gestational age exceeded 37 weeks by 91.3% whereas the control group contained a lower percentage. The results match research evidence confirming that HIE occurrences primarily affect term infants since their physiological makeup demonstrates higher metabolic activity and contrasts with preterm infant responses to hypoxic-ischemic injuries. Our findings compare with previous study regarding higher HIE vulnerability in male newborns along with two groups containing more male than female subjects. Research findings show that males become more vulnerable to HIE due to three major

factors: sex hormone variations and genetic expression levels and inflammatory response dynamics. Female neonates demonstrate increased tolerance to hypoxic-ischemic attacks because oestrogen shows protective properties in the brain.[40]

Our study agrees with multiple previous studies which tested EPO neuroprotective abilities in neonates suffering from HIE. **Ivain et al.'s** study, from five studies, 348 babies in LMICs were taken out. Only three of the five studies were able to meet the main goal of neuro-disability or death at 18 months or later. Erythropoietin reduced the incidence of neuro-disability or death at 18 months or later ($p < 0.05$) during the newborn period and during follow-up. Death or neuro-disability occurred in 27.6% of cases in the erythropoietin group and 49.7% of cases in the comparison group (risk ratio 0.56, 95% CI: 0.42–0.75).[36]

Another similar randomized controlled trial led by **Zhu et al.** showed that multiple low-dose EPO treatments reduced disability risks for infants with moderate HIE without detecting additional health complications. Positive trial findings validate EPO's potential as an effective neuroprotective therapy when medical professionals use appropriate administration dosages.[34]

The benefits and risks of EPO treatment remain unclear to scientists studying its application together with therapeutic hypothermia. The HEAL trial established that combining EPO administration with therapeutic hypothermia did not yield better safety and efficacy results than the placebo group during treatment of neonates with HIE. Serious adverse events occurred more frequently in patients who received EPO therapy according to study findings which raises important safety questions for this particular clinical application.[41]

Maternal Parity:

A total of 60.9% mothers under EPO care delivered their second or subsequent child whereas 39.1% delivered their first child. Out of all the mothers assessed in the control group 37 percent were first-time mothers. The risk assessment for moderate to severe HIE during obstetric

emergencies included maternal parity and previous caesarean delivery according to **Liljestrom et al**. Among nulliparous women the occurrence of obstetric emergencies amounted to 21% yet these emergencies appeared at a rate of 37% in parous women. Shoulder dystocia proved to be the primary obstetric emergency faced by nulliparas yet placental abruption affected parous women who had not undergone prior caesarean section and uterine rupture primarily occurred in parous women who had experienced a previous caesarean delivery.[42]

Delivery Complications:

In our study we found that in EPO group, 56.5% of babies had meconium-stained amniotic fluid, and 17.4% exhibited abnormal foetal heart sounds. While specific studies correlating these exact complications with HIE incidence are limited, it's known that such intrapartum events can compromise fetal oxygenation, increasing the risk of HIE. The study by Liljestrom et al.emphasizes the significant association between obstetric emergencies and the occurrence of HIE, suggesting that timely recognition and management of such complications are crucial.[42]

Mode of Delivery:[43]

The regular birth rate came out to 60.9 % NVD among both groups while lower segment caesarean section (LSCS) resulted in 39.1% of births. Delivery methods play an important role in limiting the development of HIE when obstetric emergencies occur. Uterine rupture manifests more often in parous women who experienced previous caesarean delivery because it establishes a strong connection to HIE incidence. According to Liljestrom et al.'s population-based cohort study of 692 428 live-born infants in Sweden at ≥ 36 gestational weeks between January 1, 2009, and December 31, 2015, uterine rupture was the most frequent obstetric emergency among women who had previously had a caesarean section (38%) and the strongest predictor of HIE among all emergencies, with a 45-fold increased risk.[42]

Resuscitation Methods:

In our study we found that infants under positive pressure ventilation therapy in the EPO group were almost twice as common as infants who needed stimulation resuscitation (63% versus 28.3%). Advanced resuscitation techniques become necessary when hypoxic disturbances prove to be serious. Recent studies demonstrate a need for positive pressure ventilation when treating HIE cases because the severity of the condition aligns with this treatment method.

Primary Respiratory Support

The study revealed no evidence of statistical difference in primary respiratory support modality distribution between EPO and Control patients ($p = 0.17$). The EPO group employed High-Flow Nasal Cannula (HFNC) as a primary respiratory support method to a greater extent than the Control group according to recorded data (69.64% and 56.5% respectively). The Control group exhibited higher usage of synchronized intermittent mandatory ventilation (SIMV) at 34.8% while the EPO group used it at 15.2%.

The present study findings match scientific literature which shows that the degree of encephalopathy requires a direct correlation with the required level of respiratory support. The invasive respiratory management method of mechanical ventilation becomes essential for patients with severe encephalopathy since their neurological impairment weakens both their ability to breathe independently and protect their airways.[40]

The evidence from this study demonstrates that EPO treatment leads to decreased encephalopathy severity which becomes apparent through both improved MRI images and decreased need for ventilation support. The observed results match published research which demonstrates that EPO provides protective benefits to the brain in newborns with HIE.[44]

The lack of facilities for therapeutic hypothermia lowers survival rates and raises morbidity rates. Several trials carried out on neonates with HIE have demonstrated that EPO is helpful in

lowering morbidity and improving neurodevelopmental outcome, even in neonates who did not receive therapeutic hypothermia .[43]

Duration of Respiratory Support:

In our study we found that 78.3% (n=36) of newborns in the EPO group required 3–7 days of respiratory support, whereas 59.5% (n=28) of the control group required 3-7 days. Whereas (6.5% vs 32.6%) newborns needed more than 7 days in the EPO group and control group respectively. This difference in duration of respiratory support was found to be statistically significant.

Zhu et al. (2009) conducted a randomized controlled trial to research the effectiveness of recombinant human erythropoietin when given to term neonates suffering from moderate to severe HIE. The research showed that administering EPO in repeated low doses to patients decreased their risk of serious disabilities without causing any noticeable adverse effects. And also, patients receiving EPO treatment exhibited better clinical outcomes that included length of time needing respiratory support among other possible factors.[34]

The research by **Pan et al. (2023)** conducted meta-analysis of EPO treatments for neonatal HIE in numerous randomized controlled trials. EPO showed positive results during assessment of its effects for patients who received HIE treatment. Data from included studies demonstrated a possible benefit of EPO treatment because participants needed less prolonged respiratory assistance based on their clinical outcome reports beyond neurodevelopmental assessment results.[43]

Occurrence of Neonatal Convulsions:

In the current study we found that EPO administration showed no relationship with neonatal convulsions occurrence. The research findings match those previously reported by **Glass et al**. in their evaluation of neonatal seizure risks between hypothermia and EPO therapy versus

placebo. Among all studied neonate's electrographic seizures developed in 31% of cases regardless of treatment with EPO or placebo. EPO treatment reduced the need for anti-seizure medications because the EPO group received medications only 36% of the time whereas the placebo group required medications in 54% of cases.[44]

Haemoglobin and HIE

In our study we found that Hb level among EPO is 16.5 mg/dl compared to control which is 17.17mg/dl and this found to be significant at admission and after 48hrs the values among EPO is 16.81 mg/dl compared to control which is 15.90 mg/dl and this found to be insignificant. Four similar studies presented haemoglobin (Hb) comparison data between the EPO and control groups (EPO group/control group = 195/196). These trials showed no discernible heterogeneity ($\chi 2 = 4.35$, $P = 0.23$, $I2 = 31.0\%$). Neonates receiving EPO medication exhibited increased Hb levels, according to the results (WMD = 1.33, $P < 0.00001$). The red blood cell (RBC) count showed a similar outcome. (EPO vs. control group, WMD = 0.51, P = 0.008) Three studies were considered in order to examine the impact of EPO on platelet (PLT) count. The EPO and control groups did not significantly vary, according to the data (OR = 1.29, 95% CI: 0.92–1.80; P = 0.14). Neonatal hypoxic-ischemic encephalopathy and erythropoietin: A revised meta-analysis of randomized control trials[43]

Neonatal seizures and EPO treatment

Our results indicated that among neonates who had seizures in the EPO intervention group showed 18.5% (n=5) abnormal resistive index (RI) measurements whereas the control group had 65.7% (n=23) such cases with a very strong statistical significance ($p < 0.001$). Laboratory data show that erythropoietin (EPO) has a protective mechanism which helps decrease cerebrovascular resistance in newborns diagnosed with hypoxic-ischemic encephalopathy (HIE).

A randomized control study done by **Wu et al. (2022)**[41] examined EPO's effectiveness when used on term neonates with HIE who received therapeutic hypothermia. Regardless of the treatment group, study results demonstrated that neonates who received EPO showed better motor skills at one year of age than subjects in the placebo group. The evidence presented though this study did not test RI measurements still supports our research by showing positive motor outcome effects that may indicate EPO's protective benefits for brain tissues.

Anti-convulsant and EPO treatment

The EPO treatment group needed less anticonvulsant drugs while experiencing fewer abnormal RI cases ($p < 0.01$). The EPO group required fewer neonates to use more than one anticonvulsant medication compared to the control group ($p < 0.07$). Patients in the EPO group needed less anticonvulsant medication before being discharged according to statistical analysis ($p < 0.01$). The EPO group needed fewer inotrope medications demonstrating stable blood pressure levels ($p < 0.01$).

Another study by **Glass et al**., in contrast to ours, revealed that 46 out of 150 neonates (31%) experienced EEG seizures (31% in EPO vs. 30% in placebo, $p = 0.96$). (Median 11.4 for Epo, IQR: 5.6, 18.1 vs. median 9.7, IQR: 4.9, 21.0 min/h for placebo) There was no discernible difference between the groups. There was no discernible difference in seizure risk or burden between the groups of neonates with HIE treated with hypothermia who were randomized to EPO or placebo. [32]

Malla et al. (2017) conducted a systematic review with meta-analysis to evaluate EPO therapy for perinatal HIE. The clinical research showed EPO treatment applied to neonates suffering from perinatal HIE leads to decreased chances of brain damage and cerebral palsy alongside reduced cognitive impairment risks. EPO's general neuroprotective action might improve blood circulation stability which potentially decreases the requirement for inotropic medications.

Amplitude Electroencephalography(aEEG) Findings.

Burst Suppression:

The EPO group experienced fewer episodes of burst suppression at 6.5% whereas the control group showed 21.7% of patients affected ($p < 0.001$).

Medical research about the impact of EPO treatment on neonatal burst suppression patterns remains scarce but shows established neuroprotective effects for EPO. The meta-analysis by **Razak A et al**. revealed EPO administration cuts down the brain damage probability among newborns who have HIE. The protective brain injury effect discovered in our study matches the results showing reduced burst suppression [45]

Continuous Background Activity:

In the EPO-treated neonates continuous background activity increased significantly to 47.8% while the control group showed only 8.7% ($p < 0.001$).

The existing research demonstrates that continuous background activity observed through CFM video monitoring leads to better neurological outcomes in newborns. The reported clinical results from **Zhu et al.** demonstrated that moderate HIE infants receiving multiple doses of low-dose EPO developed lower disability risks with no apparent side effects. Neurological improvement in the EPO-treated group directly corresponded to the higher rates of continuous background activity that were recorded.[34]

Continuous Low Voltage and Flat Trace:

Our Study found that Continuous low voltage appeared in 4.3% of EPO-treated cases while flat trace patterns occurred in 8.7% of cases but the control group displayed lower outcomes with 10.9% of continuous low voltage and 13.0% flat trace patterns ($p < 0.001$ respectively).

Studies demonstrate that the observed CFM patterns in infants represent severe brain damage which leads to unfavourable prognosis. HEAL trial results revealed that administering EPO with therapeutic hypothermia was not more effective than placebo in reducing death or neurodevelopmental impairment but it increased serious adverse event frequencies. The HEAL trial results differ from other studies because the dissimilarities between CFM patterns in your study indicate EPO may enhance selected electrophysiological outcomes.

Discontinuous Activity:

The EPO-treated group (30.4%) displayed the same level of discontinuous activity as the control group (39.1%) according to statistical assessment ($p = 0.094$).

The literature shows that moderate HIE can cause discontinuous activity which might show gradual changes throughout time.

A study by **Charki et al** showed that Out of the neonates who had asphyxia, 22% in the EPO group showed a discontinuous pattern on the aEEG, while 24% in the Control group did the same. In the EPO group, 11% showed a burst suppression pattern, 4% had a low voltage pattern, and 3% had a flat trace. On the flip side, in the Control group, 19% had a burst suppression pattern, 10% had a low voltage pattern, and 7% ended up with a flat trace. Even after rewarming, 7% still displayed a concerning prognosis pattern. In the first 24 hours of life, around 47% experienced clinical or electrical seizures.[33]

Status Epilepticus:

In comparison to the control group with 6.5% status epilepticus occurrence the EPO group presented 2.2% occurrence levels ($p < 0.001$).

Existing studies yielded no specific data on status epilepticus incidence in EPO-treated newborns but established seizure reduction as a general result from EPO administration. The

research conducted by **Glass et al.** demonstrated neonates who got EPO treatment experienced fewer electrographic seizures than controls but displayed equivalent seizure rates. Research indicates that EPO administration leads to decreased seizure intensity among HIE patients.[44]

NSG (Neurosonogram) findings

Our study shows that 15.2% of patients in the EPO-treated group displayed Abnormal RI resistive index (RI), but in the control group 56.5% displayed Abnormal RI with a significant p-value (<0.001). It was found that 84.8% of neonates in the EPO group had a normal Resistive Index (RI), compared to 43.5% (n=20) in the control group. This implies that resistive index (RI) could be protected by EPO treatment.

A similar study **by Sheikh Nisar et al** [47]shows that how cerebral RI measurements performed during the first 72 hours of life affect the short-term survival of term babies diagnosed with HIE. Hospitals reported more patient deaths within their stay for infants who had abnormal RI results (28.6%) rather than infants with normal RI (13%); these differences reached statistical significance ($p<0.01$). The research demonstrated that infants with abnormal RI at 6 months exhibited abnormal neurological results in 70.6% of cases yet normal RI assessments detected neurological problems only in 23.3% of infants ($p<0.01$). Neonates with HIE benefit from using cerebral RI as an important diagnostic tool that predicts their future development.

Another similar study by **Gowthami et al** shows that about 32 cases had grade I HIE, 8 had grade II HIE, and 14 had grade III HIE**.** The RI was abnormal in 19 cases (35.19%) and normal in 35 cases (64.81%). The RI was found to be an effective predictor of neonatal prognosis in cases of HIE.[48]

Twenty-eight instances had grade I HIE, six had grade II HIE, and ten had grade III HIE, according to a related study by **Gowthami G. S. et al.** Thirteen instances (31.8%) had an abnormal resistive index, while thirty cases (68.1%) had a normal one. HIE caused 5% of

deaths and 95% of survivors. Seizures (11.4%) and acute renal damage (34%) were the most frequent consequences. A good predictor of infant fate in cases of hypoxic-ischemic encephalopathy was discovered to be the resistant index. [49]

Our study finds that EPO administration is associated with a higher proportion of moderate encephalopathy cases and a lower proportion of severe cases.

a randomized prospective study by **Zhu et al.**[34] showed similar findings with our study that evaluation framework to examine the effectiveness with safety aspect of EPO for neonatal HIE patients. The study enrolled 167 term infants with HIE severity levels of moderate or severe who were assigned into two groups with 83 infants in the EPO treatment group and 84 infants in the conventional treatment group. At 18 months the risk of death or disability was lower in EPO-treated infants (24.6%) than control infants (43.8%) according to study results which also revealed EPO displayed no adverse effects after treatment of infants with moderate HIE.

Another similar study **Razak et al.** (2018) analysed six randomized clinical trials involving 454 neonates suffering from perinatal HIE through systematic review and meta-analysis. Laboratory tests revealed that administering EPO to infants considerably lowered their chances of developing brain injury and cerebral palsy as well as cognitive disability. EPO treatment without hypothermia provided better protection against cerebral palsy and moderate to severe cognitive impairment than placebo treatment did (relative risk [RR] 0.47, 95% confidence interval [CI] 0.27–0.80 and RR 0.49, 95% CI 0.28–0.85 respectively). EPO administration led to significantly lower brain injuries in treated infants as shown through an effect size of RR 0.70 (95% CI 0.53–0.92).[45]

In comparison with our study by **Wu et al**. (2022) operated with 501 infants who displayed moderate to severe HIE under standard therapeutic hypothermia treatments that received either EPO or placebo therapy. This research report indicated 52.5% death or neurodevelopmental

impairment occurrence in the EPO group while the placebo group experienced 49.5% death or impairment cases. Therefore, EPO treatment did not reduce the death or neurodevelopmental impairment risk rate compared to placebo. Children in the EPO treatment received more serious adverse events compared to those receiving placebo treatment.[41]

MRI Findings and HIE Neonates.

Analysis was conducted of MRI examinations on neonates with hypoxic-ischemic encephalopathy (HIE) who received human recombinant erythropoietin (EPO) therapy and the HIE patients without EPO intervention. Among infants in the EPO group MRI was not performed on 45.9% while 39.1% showed Regional specific patterns of HIE and 8.6% suffered from severe HIE and 6.5% had normal MRI results. The control group found that 45.6% of patients did not undergo MRI tests and 19.5% showed regional HIE evaluation while 32.6% showed severe HIE presentation and 2.1% experienced normal MRI results.

Experimental research **by Wu et al**. (2022) evaluated EPO therapy in neonates with HIE receiving therapeutic hypothermia in a randomized placebo-controlled trial. Years after treatment, EPO medication failed to lower mortality or developmental problems in comparison to placebo therapy at age 22–36 months. Healthcare professionals using MRI tools determined that EPO-treated infants displayed fewer instances of severe brain injury patterns. The use of EPO resulted in moderate or severe brain injury according to MRI scans in 11% of patients but the placebo group presented this outcome in 28% indicating EPO potentially served as a neuroprotective agent against severe brain injury.[41]

A group of researchers conducted the Neonatal Erythropoietin and Therapeutic Hypothermia Outcomes (NEATO) study to examine how EPO treatment impacted brain injury volume parameters as well as 1-year development outcomes in HIE newborns by **Sarah et al. 2017**

showed that infants who received EPO treatment showed lower injury volume for brain acute injuries in comparison to placebo participants according to research findings.[46]

The research indicates that erythropoietin administration reduces the likelihood of severe hypoxic-ischemic encephalopathy patterns appearing in magnetic resonance images. The research conducted by The Neonatal Erythropoietin and Therapeutic Hypothermia Outcomes project demonstrated the EPO-treated patients exhibited smaller injuries compared to placebo participants (P = .004). Neonates receiving EPO as treatment showed fewer adverse effects on their neurodevelopmental outcomes at month twelve which suggests that EPO protects the brain from severe injuries in HIE patients. [46]

Another study regarding serial MRI results and clinical outcomes from HIE patients determined deep Gray matter involvement in first MRI scans identified as a sign of poor prognosis and normal MRI results linked to better development outcomes. MRI proves to be a crucial prognostic method which helps healthcare providers determine the brain damage severity levels and future development expectations for newborns affected by HIE.

We found that in our study indicates that EPO-treated subjects showed moderate encephalopathy patterns combined with specific brain injury areas on MRIs alongside normal brain appearance but their numbers were fewer than those with severe brain injuries.

Wu et al. (2022) conducted a placebo-controlled randomized trial to determine EPO's clinical effects when treating neonates with HIE through therapeutic hypothermia. Results from MRI testing showed moderate or severe brain injury happened in 11% of patients taking EPO yet 28% of patients in the placebo group developed such injuries which indicates EPO may play a protective role against severe brain injuries. The study findings showed EPO therapy helps decrease both brain injuries as well as cerebral palsy development and cognitive damage in

newborns. The administration of EPO proved protective against brain injury as noted by a relative risk value of 0.70 within a confidence interval range from 0.53 to 0.92.[41]

[A] study **by Charki et al** shows that MRIs on 25 newborns in the EPO group and 23 in the control group. Looking at the results, we noticed some interesting trends: in terms of regional-specific HIE, 7% of the EPO group showed major findings, compared to 13% in the control group. For those with severe HIE, the percentages were a bit lower, with 4% in the EPO group and 9% in the control group.[33]

Mortality outcome

In our study we found that 97.8% discharged among cases and 89.1% among control. 1(2.17%) Death happened in cases but 10.8%(n-5) death happened among control groups and this value is found to be significant.

Another similar study by Charki et al shows that the incidence of death or neurodevelopmental impairment was 30% in the EPO group and 56.7% in the placebo group (95% CI, P = .04)[33].

Strength

1.The research demonstrates how human recombinant erythropoietin (EPO) shows promise as a substance that protects brain tissue in infants with hypoxic-ischemic encephalopathy (HIE).

2.EPO treatment led to a notable decrease of severe encephalopathy among neonates thus demonstrating positive clinical benefits.

3.MRI evidence supports the analysis of brain injuries by showing patterns between both the EPO group and control group.

4.A comparison between patient groups offered precise evidence about how EPO treatment decreased the extent of serious neurological harm.

5.The results present long-term benefits of EPO treatment that contribute to the expanding body of evidence regarding its use for HIE management.

6.MRI findings demonstrated how EPO administration decreased the frequency of severe brain injuries observed in treated neonates thus establishing its protective role towards brain health.

7.The mortality and morbidity decreased significantly because neonates treated with EPO demonstrated statistically meaningful reductions in severe encephalopathy cases which resulted in higher survival rates and reduced lifelong disabilities.

8.The results achieve stronger practical applicability when EPO treatment occurs during its critical therapeutic time frame.

9.A well-defined control group in the study enabled clear reduction confirmation of brain injury severity through EPO therapy while minimizing the effect of bias.

10.The findings displayed a strongly significant p-value that reached below 0.001 for severe HIE reduction among EPO-treated subjects validating the clinical importance of results

11.The study demonstrated that EPO treatments within the neonatal intensive care unit (NICU) represent a feasible approach because they offer practical and cost-effective implementation for standard clinical implementations.

12.The study indicates EPO therapy would work alongside therapeutic hypothermia (TH) to create the most effective neuroprotection strategy for brain protection despite its added benefits.

Limitations

1.The restricted participant numbers in the study could reduce the ability to generalize the obtained findings.

2.The research only evaluated neurodevelopmental progress through the acute stage of encephalopathy because it did not assess prolonged developmental outcomes following EPO administration.

Recommendation

1.Additional large-scale randomized clinical trials need to be performed throughout multiple centres to check the lasting neurodevelopmental effects of EPO treatment for HIE-affected newborns.

2.Medical expertise needs to create standardized protocols regarding EPO administration along with its therapeutic duration to achieve ideal results.

3.Researchers should conduct additional follow-ups to measure extended-term neurodevelopmental effects which evaluate mental and physical capabilities and social abilities of patients.

4.Medical research should investigate the potential for superior therapeutic protection by combining EPO therapy with therapeutic hypothermia procedures.

5.Researchers need to investigate potential risks associated with EPO therapy in order to guarantee the protection of neonates who receive this therapy.

Summary

Our findings align with some studies indicating that EPO administration is associated with improved outcomes in neonates with moderate HIE. However, other research suggests that EPO may significantly reduce the risk of death or neurodevelopmental impairment and may be associated with a higher rate of serious adverse events. These discrepancies highlight the need for further research to clarify the efficacy and safety of EPO in this population.

Conclusion

The research shows that it is safe and practical to provide recombinant human erythropoietin (rhEPO) to newborns suffering from moderate to severe hypoxic-ischemic encephalopathy (HIE). In comparison to the control group, the results show that rhEPO treatment considerably lowers the occurrence of abnormal resistive index (RI). In Furthermore, EPO-treated neonates showed improvements in electroencephalographic (EEG) patterns, neuro-sonograms' (NSG) resistive index and MRI brain findings, and suggesting possible neuroprotective advantages.

These findings are consistent with earlier research showing that EPO has anti-inflammatory, anti-apoptotic, and neuro-regenerative qualities that aid in the recovery of the brain after hypoxic-ischemic injury. More extensive research and long-term follow-up evaluations are necessary to confirm the effectiveness of rhEPO as a conventional therapeutic intervention in light of these encouraging results.

BIBLIOGRAPHY

1. Basinger H, Hogg JP. Neuroanatomy, brainstem.
2. Konan LM, Reddy V, Mesfin FB. Neuroanatomy, Cerebral Blood Supply.
3. Maldonado KA, Alsayouri K. Physiology, brain. InStatPearls [Internet] 2023 Mar 17. StatPearls Publishing.
4. Greco P, Nencini G, Piva I, Scioscia M, Volta CA, Spadaro S, Neri M, Bonaccorsi G, Greco F, Cocco I, Sorrentino F. Pathophysiology of hypoxic–ischemic encephalopathy: a review of the past and a view on the future. Acta Neurologica Belgica. 2020 Apr; 120:277-88.
5. Banu SH, Salim AF, Ara R, Akhter R, Khan NZ. Neurodevelopmental evaluation in full-term newborns with neonatal hypoxic ischemic encephalopathy (HIE): a case control study. Bangladesh Journal of Child Health. 2015;39(1):6-13.
6. Douglas-Escobar M, Weiss MD. Hypoxic-ischemic encephalopathy: a review for the clinician. JAMA pediatrics. 2015 Apr 1;169(4):397-403.
7. Harteman JC, Nikkels PG, Benders MJ, Kwee A, Groenendaal F, de Vries LS. Placental pathology in full-term infants with hypoxic-ischemic neonatal encephalopathy and association with magnetic resonance imaging pattern of brain injury. The Journal of pediatrics. 2013 Oct 1;163(4):968-75.
8. Wassink G, Gunn ER, Drury PP, Bennet L, Gunn AJ. The mechanisms and treatment of asphyxial encephalopathy. Frontiers in neuroscience. 2014 Feb 27; 8:40.
9. Greco P, Nencini G, Piva I, Scioscia M, Volta CA, Spadaro S, Neri M, Bonaccorsi G, Greco F, Cocco I, Sorrentino F. Pathophysiology of hypoxic–ischemic encephalopathy: a review of the past and a view on the future. Acta Neurologica Belgica. 2020 Apr; 120:277-88.

10. Ludwig PE, Reddy V, Varacallo MA. Neuroanatomy, neurons. InStatPearls [Internet] 2023 Jul 24. StatPearls Publishing
11. Konan LM, Reddy V, Mesfin FB. Neuroanatomy, Cerebral Blood Supply.
12. Gawdi R, Emmady P. Physiology, blood brain barrier. StatPearls. 2020 Sep 27.Ba
13. Nelson, R. M., & Behrman, R. E. (2017). Nelson's textbook of paediatrics (21st ed.). Elsevier India.
14. Gupta, Piyush. PG Textbook of Pediatrics. 3rd ed., Elsevier India, 2019.
15. Chavez-Valdez R, Martin LJ, Northington FJ. Programmed Necrosis: A Prominent Mechanism of Cell Death following Neonatal Brain Injury. Neurol Res Int. 2012; 2012:257563.
16. Chaabane W, User SD, El-Gazzah M, Jaksik R, Sajjadi E, Rzeszowska-Wolny J, et al. Autophagy, apoptosis, mitoptosis and necrosis: interdependence between those pathways and effects on cancer. Arch Immunol Ther Exp (Warsz). 2013; 61(1):43–58.
17. Sánchez-Rodríguez EC, López VJ. Hypoxic ischemic encephalopathy (HIE). Frontiers in Neurology. 2024 Jul 23; 15:1389703. Roto S, Nupponen I, Kalliala I, Kaijomaa M. Risk factors for neonatal hypoxic ischemic encephalopathy and therapeutic hypothermia: a matched case-control study. BMC Pregnancy and Childbirth. 2024 Jun 12;24(1):421.
18. Butt TK, Farooqui R, Khan MA. Risk factors for hypoxic ischemic encephalopathy in children. J Coll Physicians Surg Pak. 2008 Jul 1;18(7):428-32.
19. Douglas-Escobar M, Weiss MD. Hypoxic-ischemic encephalopathy: a review for the clinician. JAMA pediatrics. 2015 Apr 1;169(4):397-403.
20. Risk Factors and Short Term Outcome of Hypoxic Ischemic Encephalopathy in Term Neonates with Perinatal Asphyxia

21. Sumo roto
22. Sarnat HB, Sarnat MS. Neonatal encephalopathy following fetal distress: a clinical and electroencephalographic study. Archives of neurology. 1976 Oct 1;33(10):696-705.
23. Goergen SK, Ang H, Wong F, Carse EA, Charlton M, Evans R, Whiteley G, Clark J, Shipp D, Jolley D, Paul E. Early MRI in term infants with perinatal hypoxic–ischaemic brain injury: Interobserver agreement and MRI predictors of outcome at 2 years. Clinical radiology. 2014 Jan 1;69(1):72-81.
24. Rutherford, Mary, et al. "MRI of perinatal brain injury." Pediatric radiology 2010;40(6): 819-833.
25. Awal MA, Lai MM, Azemi G, Boashash B, Colditz PB. EEG background features that predict outcome in term neonates with hypoxic ischaemic encephalopathy: A structured review. Clinical Neurophysiology. 2016 Jan 1;127(1):285-96.
26. Zhao M, Zhu P, Fujino M, Zhuang J, Guo H, Sheikh I, Zhao L, Li XK. Oxidative stress in hypoxic-ischemic encephalopathy: molecular mechanisms and therapeutic strategies. International journal of molecular sciences. 2016 Dec 10;17(12):2078.
27. Lv H, Wang Q, Wu S, Yang L, Ren P, Yang Y, Gao J, Li L. Neonatal hypoxic ischemic encephalopathy-related biomarkers in serum and cerebrospinal fluid. Clinica chimica acta. 2015 Oct 23;450:282-97.
28. Barkovich AJ, Miller SP, Bartha A, Newton N, Hamrick SE, Mukherjee P, Glenn OA, Xu D, Partridge JC, Ferriero DM, Vigneron DB. MR imaging, MR spectroscopy, and diffusion tensor imaging of sequential studies in neonates with encephalopathy. American Journal of Neuroradiology. 2006 Mar 1;27(3):533-47.

29. Filippi L, La Marca G, Fiorini P, Poggi C, Cavallaro G, Malvagia S, Pellegrini-Giampietro DE, Guerrini R. Topiramate concentrations in neonates treated with prolonged whole body hypothermia for hypoxic ischemic encephalopathy. Epilepsia. 2009 Nov;50(11):2355-61

30. Oorschot DE, Sizemore RJ, Amer AR. Treatment of neonatal hypoxic-ischemic encephalopathy with erythropoietin alone, and erythropoietin combined with hypothermia: history, current status, and future research. International journal of molecular sciences. 2020 Feb 21;21(4):1487.

31. Ezenwa B, Ezeaka C, Fajolu I, Ogbenna A, Olowoyeye O, Nwaiwu O, Opoola Z, Olorunfemi G. Impact of Erythropoietin in the management of Hypoxic Ischaemic Encephalopathy in resource-constrained settings: protocol for a randomized control trial. BMC neurology. 2020 Dec;20:1-8.

32. Glass HC, Ferriero DM. Treatment of hypoxic-ischemic encephalopathy in newborns. Current treatment options in neurology. 2007 Nov;9(6):414-23.

33. Charki S, Patil SV, Vijayakumar S, Kolkar Y. Erythropoietin in Neonates with Perinatal Asphyxia Undergoing Therapeutic Hypothermia—A Prospective Cohort Study. Journal of Neonatology. 2024:09732179231194455.

34. Zhu C, Kang W, Xu F, Cheng X, Zhang Z, Jia L, Ji L, Guo X, Xiong H, Simbruner G, Blomgren K. Erythropoietin improved neurologic outcomes in newborns with hypoxic-ischemic encephalopathy. Pediatrics. 2009 Aug 1;124(2):e218-26.

35. Bang SJ, Lee J, Jeon GW, Jun YH. Erythropoietin Reduces Death and Neurodevelopmental Impairment in Neonatal Hypoxic-Ischemic Encephalopathy. Neonatal Medicine. 2022 Nov 30;29(4):123-9.

36. Ivain P, Montaldo P, Khan A, Elagovan R, Burgod C, Morales MM, Pant S, Thayyil S. Erythropoietin monotherapy for neuroprotection after neonatal encephalopathy in low-to-middle income countries: a systematic review and meta-analysis. Journal of Perinatology. 2021 Sep;41(9):2134-40.

37. Tagin MA, Woolcott CG, Vincer MJ, Whyte RK, Stinson DA. Hypothermia for neonatal hypoxic ischemic encephalopathy: an updated systematic review and meta-analysis. Archives of pediatrics & adolescent medicine. 2012 Jun 1;166(6):558-66

38. Oorschot DE, Sizemore RJ, Amer AR. Treatment of neonatal hypoxic-ischemic encephalopathy with erythropoietin alone, and erythropoietin combined with hypothermia: history, current status, and future research. International journal of molecular sciences. 2020 Feb 21;21(4):1487

39. Perrone S, Lembo C, Gironi F, Petrolini C, Catalucci T, Corbo G, Buonocore G, Gitto E, Esposito SM. Erythropoietin as a neuroprotective drug for newborn infants: ten years after the first use. Antioxidants. 2022 Mar 28;11(4):652.

40. Ezenwa B, Ezeaka C, Fajolu I, Ogbenna A, Olowoyeye O, Nwaiwu O, Opoola Z, Olorunfemi G. Impact of Erythropoietin in the management of Hypoxic Ischaemic

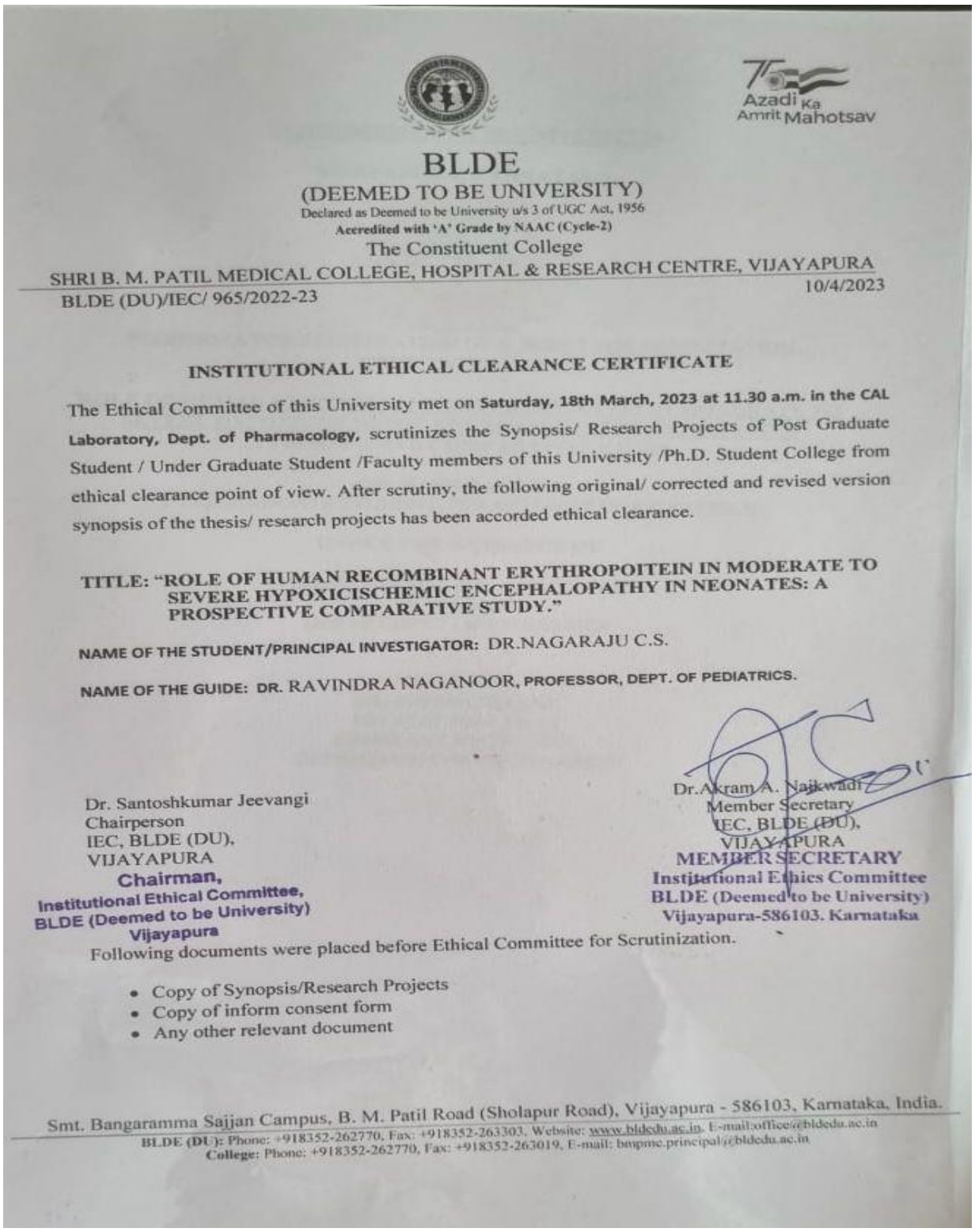
Encephalopathy in resource-constrained settings: protocol for a randomized control trial. BMC neurology. 2020 Dec;20:1-8.

41. Wu YW, Bauer LA, Ballard RA, Ferriero DM, Glidden DV, Mayock DE, Chang T, Durand DJ, Song D, Bonifacio SL, Gonzalez FF. Erythropoietin for neuroprotection in neonatal encephalopathy: safety and pharmacokinetics. Pediatrics. 2012 Oct 1;130(4):683-91.
42. Liljestrom L, Wikstrom AK, Jonsson M. Obstetric emergencies as antecedents to neonatal hypoxic ischemic encephalopathy, does parity matter?. Acta obstetricia et gynecologica Scandinavica. 2018 Nov;97(11):1396-404.
43. Pan JJ, Wu Y, Liu Y, Cheng R, Chen XQ, Yang Y. The effect of erythropoietin on neonatal hypoxic-ischemic encephalopathy: An updated meta-analysis of randomized control trials. Frontiers in Pediatrics. 2023 Jan 9;10:1074287.
44. Glass HC, Wusthoff CJ, Comstock BA, Numis AL, Gonzalez FF, Maitre N, Massey SL, Mayock DE, Mietzsch U, Natarajan N, Sokol GM. Risk of seizures in neonates with hypoxic-ischemic encephalopathy receiving hypothermia plus erythropoietin or placebo. Pediatric research. 2023 Jul;94(1):252-9.
45. Razak A, Hussain A. Erythropoietin in perinatal hypoxic-ischemic encephalopathy: a systematic review and meta-analysis. Journal of Perinatal Medicine. 2019 May 27;47(4):478-89
46. Mulkey SB, Ramakrishnaiah RH, McKinstry RC, Chang T, Mathur AM, Mayock DE, Van Meurs KP, Schaefer GB, Luo C, Bai S, Juul SE. Erythropoietin and brain magnetic resonance imaging findings in hypoxic-ischemic encephalopathy: volume of acute brain injury and 1-year neurodevelopmental outcome. The Journal of pediatrics. 2017 Jul 1;186:196-9.

47. Ahmad SN, Mehraj J, Ahmad M, Beigh MS, Mir OA, Mir NY. Prognostic value of resistive index (measured in anterior cerebral artery) in term neonates with hypoxic ischemic encephalopathy. Int J Contemp Pediatr. 2024 May;11:557-60.
48. Gowthami GS, Yeli RK, Nimbal V, Dhanya SB, Kumar P. Early Morbidities of Hypoxia-Ischemic Encephalopathy in Term Neonates With a Resistive Index as a Prognostic Indicator. Cureus. 2024 Jun 8;16(6).
49. Gowthami GS, Patil MM, Ravi Kumar Yeli KS, Patil SV. Resistive index (RI) as a prognostic indicator of early morbidities in term neonates with hypoxic ischemic encephalopathy.
50. Malla RR, Asimi R, Teli MA, Shaheen F, Bhat MA. Erythropoietin monotherapy in perinatal asphyxia with moderate to severe encephalopathy: a randomized placebo-controlled trial. Journal of Perinatology. 2017 May;37(5):596-601.

ANNEXTURE-1

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE



BLDE
(DEEMED TO BE UNIVERSITY)
Declared as Deemed to be University u/s 3 of UGC Act, 1956
Accredited with 'A' Grade by NAAC (Cycle-2)
The Constituent College
SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPURA

BLDE (DU)/IEC/ 965/2022-23 10/4/2023

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this University met on **Saturday, 18th March, 2023 at 11.30 a.m. in the CAL Laboratory, Dept. of Pharmacology,** scrutinizes the Synopsis/ Research Projects of Post Graduate Student / Under Graduate Student /Faculty members of this University /Ph.D. Student College from ethical clearance point of view. After scrutiny, the following original/ corrected and revised version synopsis of the thesis/ research projects has been accorded ethical clearance.

TITLE: "ROLE OF HUMAN RECOMBINANT ERYTHROPOITEIN IN MODERATE TO SEVERE HYPOXICISCHEMIC ENCEPHALOPATHY IN NEONATES: A PROSPECTIVE COMPARATIVE STUDY."

NAME OF THE STUDENT/PRINCIPAL INVESTIGATOR: DR.NAGARAJU C.S.

NAME OF THE GUIDE: DR. RAVINDRA NAGANOOR, PROFESSOR, DEPT. OF PEDIATRICS.

Dr. Santoshkumar Jeevangi
Chairperson
IEC, BLDE (DU),
VIJAYAPURA
Chairman,
Institutional Ethical Committee,
BLDE (Deemed to be University)
Vijayapura

Dr.Akram A. Najkwadi
Member Secretary
IEC, BLDE (DU),
VIJAYAPURA
MEMBER SECRETARY
Institutional Ethics Committee
BLDE (Deemed to be University)
Vijayapura-586103. Karnataka

Following documents were placed before Ethical Committee for Scrutinization.

- Copy of Synopsis/Research Projects
- Copy of inform consent form
- Any other relevant document

Smt. Bangaramma Sajjan Campus, B. M. Patil Road (Sholapur Road), Vijayapura - 586103, Karnataka, India.
BLDE (DU): Phone: +918352-262770, Fax: +918352-263303, Website: www.bldedu.ac.in, E-mail:office@bldedu.ac.in
College: Phone: +918352-262770, Fax: +918352-263019, E-mail: bmpmc.principal@bldedu.ac.in

ANNEXTURE-II

RESEARCH INFORMED CONSENT FORM

BLDEA's Shri B.M. PATIL Medical College, Hospital & Research Centre,
Vijayapura, Karnataka -586103.

TITLE OF THE PROJECT:

ROLE OF HUMAN RECOMBINANT ERYTHROPOIETIN IN MODERATE TO SEVERE HYPOXIC-ISCHEMIC ENCEPHALOPATHY IN NEONATES
A PROSPECTIVE COMPARATIVE STUDY

GUIDE: **DR. RAVINDRA NAGANOOR MD**
PROFESSOR,
DEPARTMENT OF PEDIATRICS

CO GUIDE: DR SIDDU CHARKI
MD, FIAP NEONATOLOGY
ASSOCIATE PROFESSOR
DEPARTMENT OF PAEDIATRICS

PG STUDENT: **DR. NAGARAJU C S**

I HAVE EXPLAINED ABOUT THE RESEARCH IN THE LOCAL LANGUAGE.

PURPOSE OF RESEARCH:

To assess and evaluate the role of rh EPO in moderate to severe HIE in neonates

PROCEDURE:

I understand that after having obtained a detailed clinical history, thorough clinical examination, and relevant investigations, a final workup of the process and its outcome planned

RISK AND DISCOMFORTS:

I understand that I may experience pain and discomfort during the examination or my treatment. It is mainly the result of my condition, and the procedures of this study are not expected to exaggerate these feelings, which are associated with the usual course of treatment.

BENEFITS:

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

CONFIDENTIALITY:

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

REQUEST FOR MORE INFORMATION:

DR. NAGARAJU C S at the department of pediatrics is available to answer my questions or concerns. I understand that I will be informed of any significant new findings discovered during the study, which might influence my continued participation. I will be given a copy of this consent form to keep for careful reading.

REFUSAL OR WITHDRAWAL OF PARTICIPATION:

I understand that my participation in this study is voluntary and that I may refuse to participate or withdraw consent and discontinue participation in the study at any time without prejudice. I

also understand that DR. NAGARAJU C S may terminate my participation in the study after they have explained the reasons for doing so.

INJURY STATEMENT:

I understand that in the unlikely event of injury to my child resulting directly from the child's participation in this study, if such damage were reported promptly, the appropriate treatment would be available to the child. But, no further compensation would be provided by the hospital. I understand that by agreeing to participate in this study and not waiving any of my legal rights.

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

We confirm that DR. NAGARAJU C S is doing a study on ROLE OF HUMAN RECOMBINANT ERYTHROPOITEIN IN MODERATE TO SEVERE HIE IN NEWBORNS admitted To NICU In Shri B. M. Patil Medical College Hospital, Vijayapura, Karnataka. NAGARAJU C S has explained to us the purpose of research and the study procedure. We will allow our child to get treated in Shri B.M. Patil Medical College Hospital, Vijayapura. We have explained the study, benefits, and possible discomforts in detail in our native language, and we understand the same.

We know that the child will get the best and standard treatment, and no compensation like financial benefits will be given if our child's condition deteriorates. Any untoward complication happens, and we will not sue anyone regarding this. Therefore we agree to provide our full consent for the child's participation as a subject in this research project.

_____

_____

DR NAGARAJU C S **DATE:**

(INVESTIGATOR)

PARENTS / GUARDIAN CONSENT STATEMENT

(Parents / Guardian)

We confirm that DR. NAGARAJU C S is doing a study on role of human recombinant EPO IN moderate to severe Hypoxic Ischemic Encephalopathy IN newborns admitted To NICU In Shri B. M. Patil Medical College Hospital, Vijayapura, Karnataka. NAGARAJU C S has explained to us the purpose of research and the study procedure. We will allow our child to get treated in Shri B.M. Patil Medical College Hospital, Vijayapura. We have explained the study, benefits, and possible discomforts in detail in our native language, and we understand the same. We know that the child will get the best and standard treatment, and no compensation like financial benefits will be given if our child's condition deteriorates. Any untoward complication happens, and we will not sue anyone regarding this. Therefore, we agree to provide our full consent for the child's participation as a subject in this research project.

_____

(Witness to signature)

ANNEXURE – III

PROFORMA OF CASE TAKING:

- NAME:
- AGE:
- SEX:
- IP NO. :
- ADDRESS:
- DATE OF BIRTH:
- DATE OF ADMISSION:
- AGE AT ADMISSION:
- DATE OF DISCHARGE:
- GESTATIONAL AGE:
- PARITY:
- GRBS AT TIME OF ADMISSION:
- MATERNAL HISTORY:
- MOTHER'S BLOOD GROUP:
- H/O ANY RISK FACTORS:
- BIRTH ORDER:
- DELIVERY AT :OUTBORN
- DATE & TIME OF DELIVERY :
- MODE OF DELIVERY:
- BIRTH WEIGHT
- ANY RESUSCITATIVE MEASURES REQUIRED:
- DRUG ADMINISTRATION DETAILS:
- PRIMARY RESPIRATORY SUPPORT:
- DURATION OF RESPIRATORY SUPPORT:
- NEED FOR VENTILATION:
- DURATION OF VENTILATION
- CONVULSIONS DURING TREATMENT:
- FIO2 REQUIREMENT:
- DURATION OF NICU STAY
- INITIATION OF EXPRESSED MILK/DBF:

- OUTCOME (DISCHARGE/DEATH):
- DATE OF DISCHARGE / DEATH:

INVESTIGATIONS:

At admission **3 rd day of admission.**

Date:

- Hb-
- PCV-
- TC-
- N/L/E/M-
- PLT-
- RBC
- BLOOD GAS ANALYSIS:

  AT ADMISSION -

  DATE:

- PH-
- PCO2-
- PO2-
- HCO3-
- BASE DEFICIT-
- LACTATE-

CRP –

- S. CREATININE –
- Na-
- K-
- Ca-
- CFM findings:
- NSG FINDINGS:

- IMAGING (MRI):

SARNAT STAGING

Level of consciousness	Stage 1 (mild) Hyperalert	Stage 2 (moderate) Lethargic/obtunded	Stage 3 (severe) Stuporous
Neuromuscular control			
Muscular tone	Normal	Mild hypotonia	Flaccid
Posture	Mild distal flexion	Strong distal flexion	Intermittent decerebration
Stretch	Overactive	Overactive	Decreased/absent
Segmental myoclonus	Present	Present	Absent
Complex reflexes			
Suck	Weak	Weak/absent	Absent
Moro	Strong	Weak	Absent
Oculovestibular	Normal	Overactive	Weak/absent
Tonic neck	Slight	Strong	Absent
Autonomic function			
Pupils	Mydriasis	Miosis	Variable
Heart rate	Tachycardia	Bradycardia	Variable
Bronchial/salivary secretions	Sparse	Profuse	Variable
Gastrointestinal motility	Normal/decreased	Increased/diarrhea	Variable
Seizures	None	Common/focal or multifocal	Uncommon
EEG	Normal/decreased	Early low voltage continuous delta and theta, later periodic, seizues focal 1–1.5 Hz spike-wave	Early periodic pattern with isopotential phases, later isopotential
Duration	<24 h	2–14 days	Hours–weeks

EEG, electroencephalogram.

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RECOMBINANT HUMAN ERYTHROPOIETIN STUDY

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ANNEXTURE-IV

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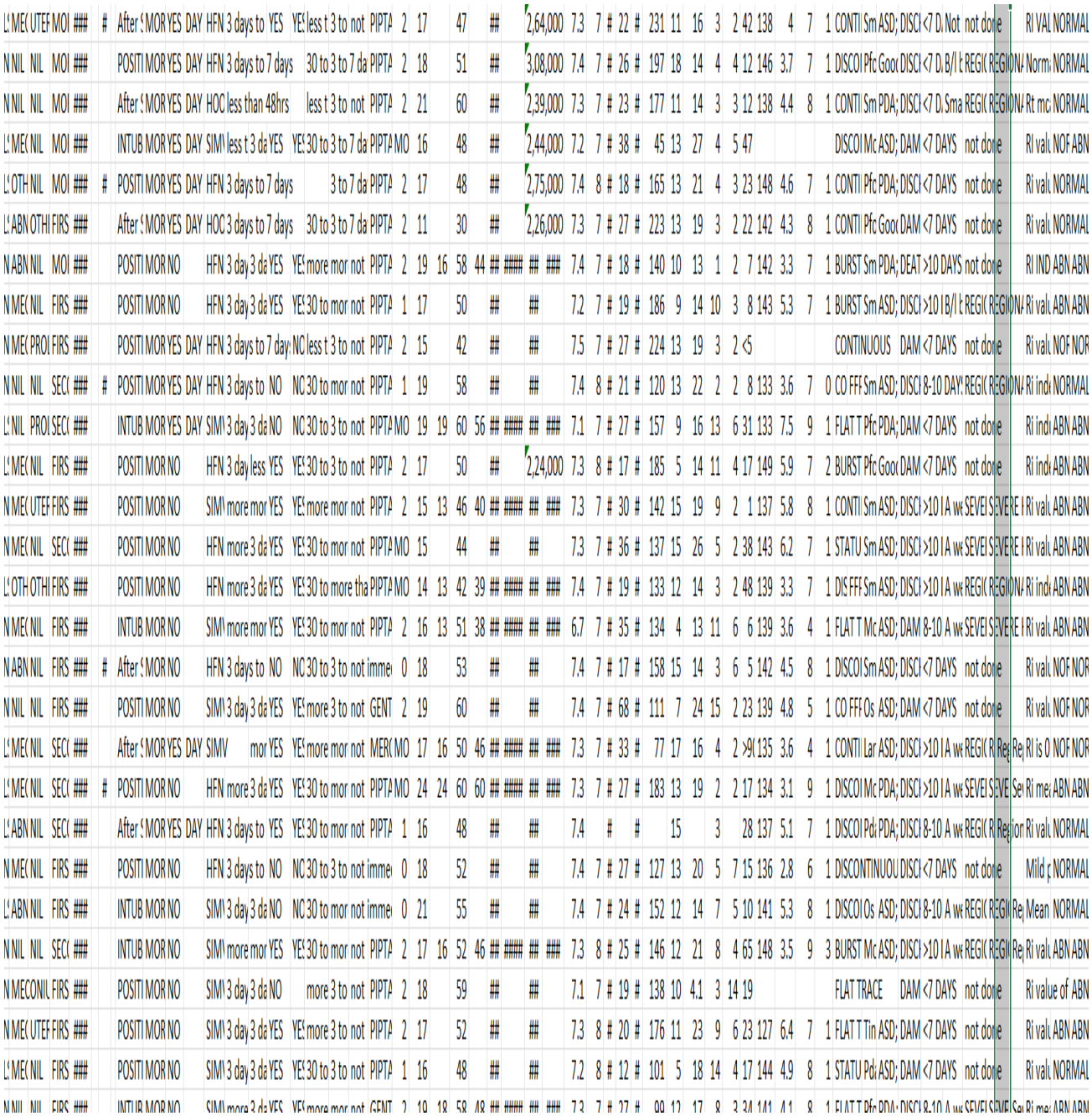

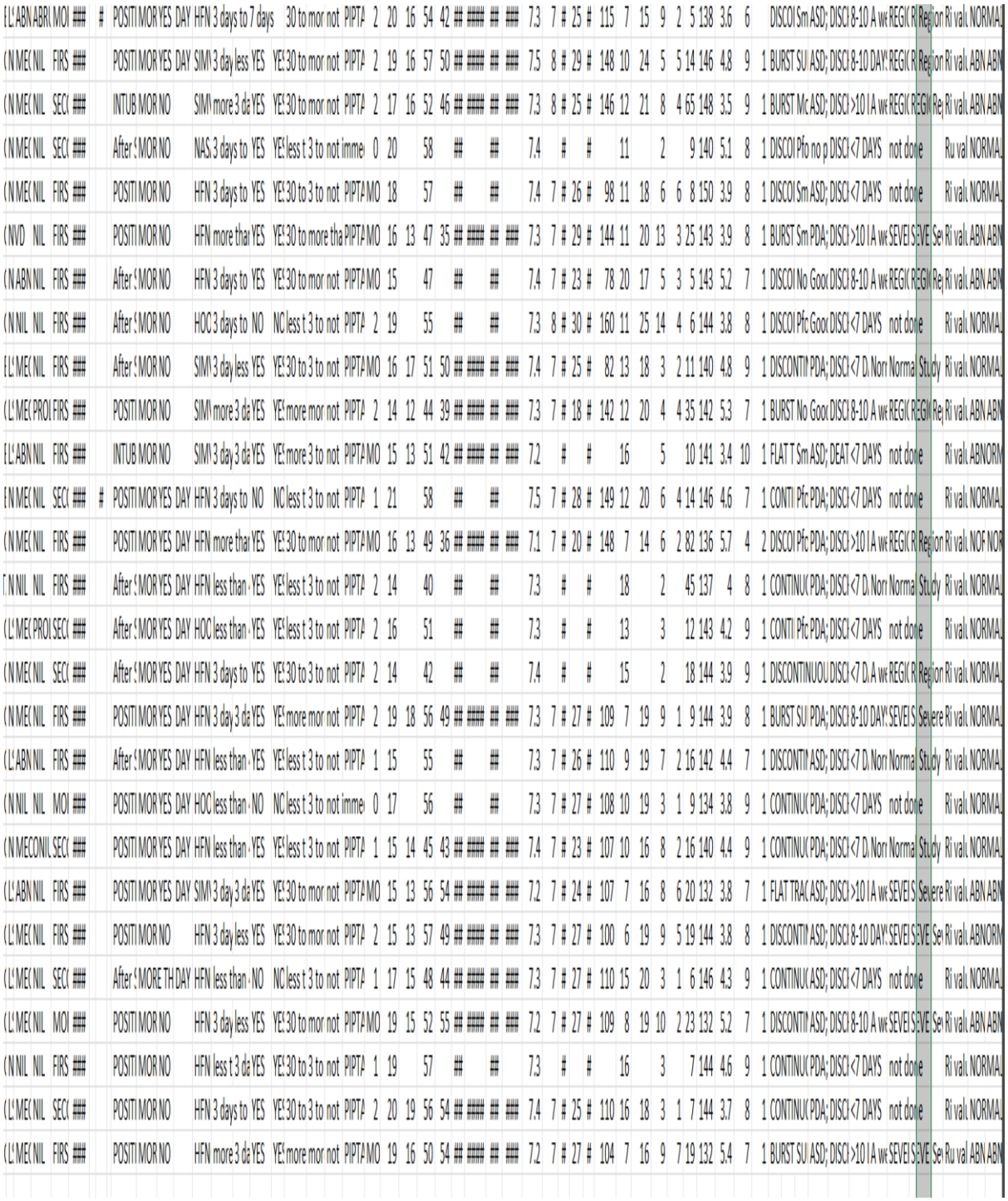

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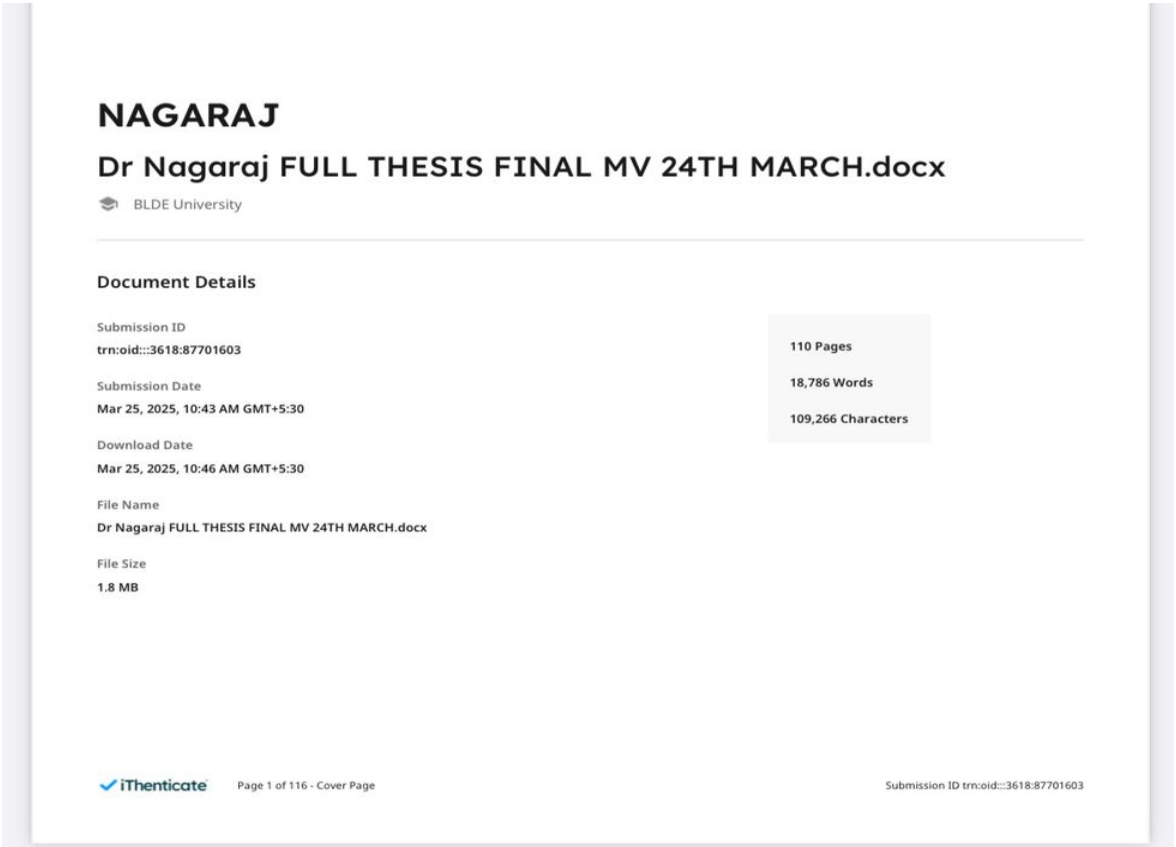

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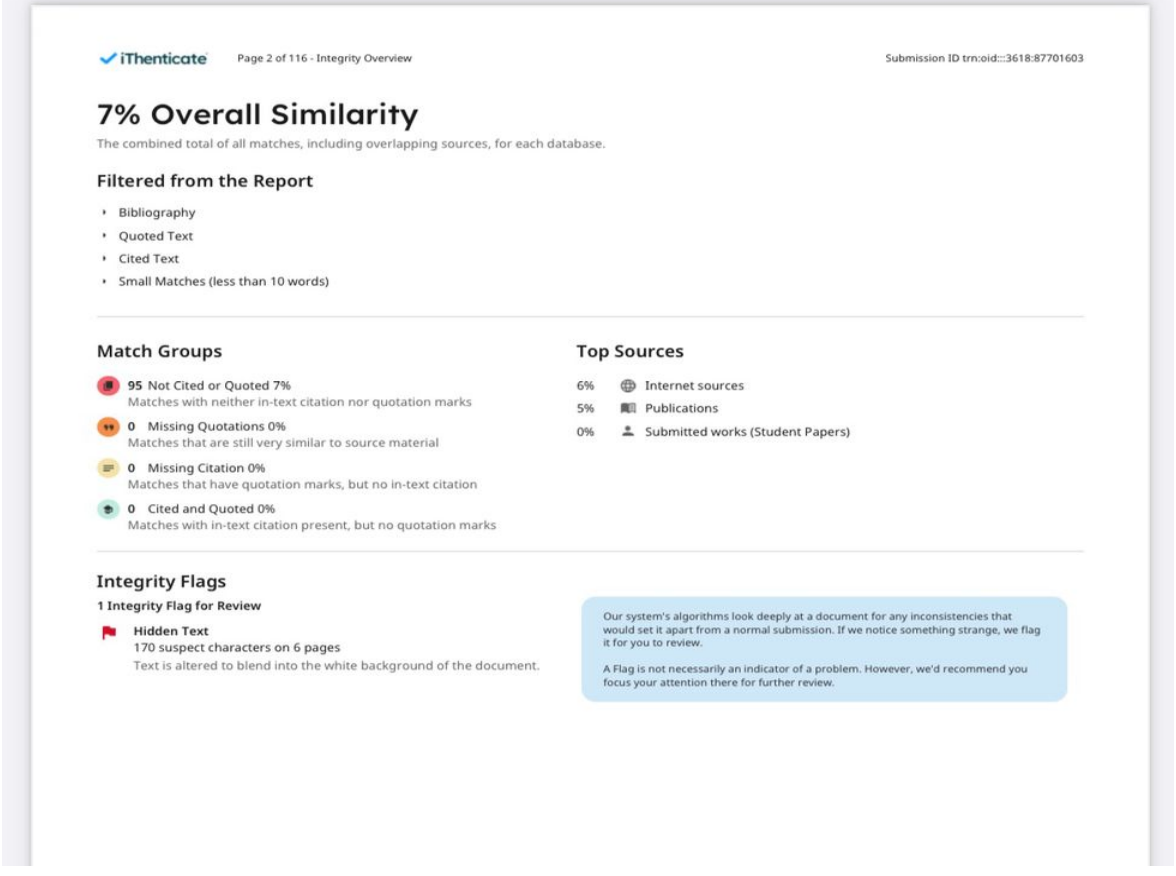

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