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Hypoxic-Ischemic Encephalopathy (HIE): Diagnostic, And Therapeutic Strategies

Clinical review

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Abstract – Hypoxic-ischemic encephalopathy (HIE) is one of the main causes of morbidity and mortality in neonates. On account of high groupings of sensitive immature cells, metal-catalyzed free radicals, non-saturated fatty acids, and low concentrates of antioxidants enzymes, the brain requires elevated degrees of oxygen supply and is, in this manner, very sensitive to hypoxia. Solid proof shows that oxidative stress assumes a significant part in pathogenesis and progression. Following hypoxia and ischemia, reactive oxygen species (ROS) production rapidly increments and overpowers antioxidant defences. A large excess of ROS will straightforwardly change or degenerate cell macromolecules, like membranes, proteins, lipids, and DNA, and lead to a cascading inflammatory reaction, and protease secretion. These derivatives are engaged with a complex interplay of numerous pathways (e.g., inflammation, apoptosis, autophagy, and necrosis) which at last lead to brain injury. In this review, we feature the molecular mechanism for oxidative stress in HIE, sum up current research on therapeutic methodologies used in combating oxidative stress, and attempt to explore novel potential clinical methodologies.

Keywords – oxidative stress, hypoxic-ischemic encephalopathy, cell damage, diagnostic strategies, therapeutic strategies.

I. INTRODUCTION

Hypoxic-ischemic encephalopathy (HIE), which is prompted by the interruption of cerebral blood flow and a subsequent lack of oxygen to the affected area, is one of the main causes of expanded risk of death and long lasting incapacity, like visual impairment, learning debilitation, epilepsy, mental retardation, blindness, and cerebral paralysis [1]. The prevention and therapy of this disease stays a severe clinical issue with worldwide financial repercussions. One of the most broadly acknowledged pathophysiological components of HIE includes the generation of oxidative stress. In light of high concentrations of sensitive immature cells, metal-catalyzed free radicals, non-saturated fatty acids, and low concentrations of antioxidant enzymes, the brain requires elevated degrees of oxygen supply and is, in this way, very sensitive to hypoxia [2]. Following the hypoxic-ischemic event, oxidative stress, which later triggers the release of oxygen and nitrogen species, calcium over-loading, free radical generation, excitotoxicity, acidotoxicity, ionic imbalance, inflammation, apoptosis, autophagy, and necrosis, assumes a significant role in pathogenesis [3].

Solid proof demonstrates that when the delicate balance between pro oxidants and anti-oxidants tips toward a more oxidative state, local reactive oxygen species (ROS) creation is dramatically increased [4]. If available in fitting sums, ROS have been displayed to act as signal transduction molecules giving cell protection and keep up with in homeostasis. Conversely, while surpassing the buffering limit of scavenging molecules and cell antioxidant enzymes, ROS are displayed to make significant damage to biological macromolecules, like proteins (membrane protein degeneration), lipids (lipid oxidation), and nucleic acids (DNA degeneration). These derivatives induce a complicated interplay of multiple pathways including immune defence, cell

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signaling, and induction of mitogenesis. In this review, we feature the molecular mechanism for oxidative stress in HIE and analyze potentially powerful therapeutic strategies [5,6].

1. Outline for ROS Formation, Decomposition, and Sources

The principal arrangement and decomposition of ROS has been shown previously [6]. In human tissues, sources of ROS production incorporate the NADPH oxidases (NOX), xanthine oxidize (XO), arachidonic acid metabolism pathways (12/15 lipoxygenase), uncoupled nitric oxide synthase (NOS), and the mitochondrial electron transport system. An enormous collection of proof recommends that NOX and NOS, alongside an oxygen-starved mitochondrial electron transport system, contain the significant sources of ROS in the brain during hypoxia and ischemia [7,8,9,10,11,12,13,14,15,16].

After a hypoxic-ischemic, resident immune cells in the brain are stimulated. They create several oxygen free radicals and afterward induce the expression of proinflammatory mediators [17]. These superoxide (counting superoxide anions ($O2\cdot-$), hydrogen peroxide (H2O2), hypochlorous acid and hydroxyl radicals ($\cdot OH$) $O2\cdot-$) are created through a several enzyme systems, including; glutathione peroxidase (GPX), cyclooxygenase (COX), xanthine dehydrogenase, XO, monoamine oxidase (MAO), NADPH oxidase and myeloperoxidase (MPO). In any case, the role of NADPH oxidase, the main important source of ROS, in neonatal inflammatory reactions following hypoxic-ischemic attack is dubious. From one viewpoint, inhibition of NADPH oxidase expands the level of inflammation cytokines [18], in any case, in vivo studies have demonstrated the way that it can exacerbate inflammatory reactions and worsens neurological results in animal models [18,19].

2. Potential Biomarkers and Clinical Manifestations for Hypoxic-Ischemic Encephalopathy (HIE)

During and after exposure to HIE, neural imaging, electroencephalography (EEG), and biochemical markers have been utilized to asses' prognosis and predict long term outcomes, like visual impairment, learning impairment, epilepsy, mental retardation, blindness, cerebral palsy, and even death. As the range of imaging findings relates to the development of ischemic parenchymal tissue (basal ganglia and thalami, corticospinal tract, white matter, and cortex), magnetic resonance image (MRI) is the favoured imaging choice. Doppler sonography is additionally sensitive in the identification of HIE. In light of the great water content in the cerebrum, and high protein content of the cerebrospinal liquid, which brings about unfortunate parenchyma contrast goal, registered tomography (CT) is the least sensitive methodology for the assessment of HIE [20,21,22,23]. Like imaging methods, EEG can be promptly estimated at the bedside. The most encouraging EEG highlights in distinguishing HIE incorporate burst suppression, low voltage, and a flat trace [24]. Various numbers of biochemical markers in body fluids have likewise been proposed to be helpful as sentinel biomarkers, including 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, IL-6, IL-8, and IL-1β [20,25,26,27].

3. Pathogenesis and Molecular Mechanisms

3.1. Inflammation Mediated Oxidative Stress in HIE

Both clinical and trial studies propose that oxidative stress assumes a part in crosstalk between inflammatory systems and makes "windows of susceptibility" to HIE. Two periods of HIE-induced oxidative stress mediated by inflammatory reactions have been recognized. In the initial step, inflammation is actuated by amoeboid microglia, the resident immune cell in the brain. Microglia respond vigorously to hypoxic-ischemic attack and produce excess inflammatory cytokines (IL-1β, TNF-α, and so forth) alongside glutamate, nitric oxide (NO), and ROS [28,29]. Close by microglia, astrocytes are likewise activated by ROS, and secret proinflammatory cytokines (IL-6, TNF-α, IL-1α, and IL-1β) and IFN-γ. Rapid increases in the levels of these cytokines cause collective accumulation in the brain tissue, prompting direct injury through expanding levels of toxic NO, inducing the apoptosis of neuronal cells, restraining neurogenesis, and attracting in immune cells to the ischemic site [30]. Brain tissue injury is exacerbated by the complex interaction between neutrophils, lymphocytes, neutrophils, adhesion molecules, cytokines, and chemokines. During ischemia, neutrophils can enhance brain injury through inducing ROS production. Interestingly, nonetheless, studies have shown that lymphocytes assume a negative part in the pathogenesis of the acute ischemic brain [31,32,33]. The main cytokines relating with the inflammatory reactions seen in HIE are IL-1, IL-6, IL-10, TNF-α, and TGF-β, with elevated levels of these cytokines under oxidative stress correlating positively with HIE severity [34,35,36,37,38].

3.2. Mitochondrial Injury in HIE

Mitochondria function as "power houses", producing adenosine triphosphate (ATP), and act as the major sites of oxidative metabolism [39,40]. During HIE, oxidative stress brought about by ROS "bursts" assumes a significant part in changes in the mitochondria, including ischemic starvation, reperfusion-prompted hyper activation, mitochondrial dysfunction, and delayed neuronal death [41]. At first, during oxidation related with mitochondrial respiration, the mitochondrial membrane potential ($\Delta\psi m$) becomes positive in the inner chamber, rather than physiological conditions. Besides, there is an increment of intracellular Ca2+, with diminishing glucose and ATP generation, and cytochrome c (CytC) releases into the cytoplasm. During HIE, the release of CytC from the mitochondrial serves as a fairly significant pathway for the cascade of apoptotic events. In the first place, approximately coupled or firmly bound CytC is damaged in the mitochondrial membrane and then released; second, apoptotic proteins, Bid, Bad, Bax, Bak, Bok, and Bim in the outer mitochondrial layer increment the permeability of the membrane, forming explicit pores and stimulating free CytC release; third, CytC binds to apoptosis protein-associated factor 1 (Apaf-1) and forms the Apaf-1/caspase-9/CytC complex. At long last, caspase-3 is activated, which triggers apoptosis and delayed neuronal death [42,43].

3.3. Oxidative Stress Mediated Apoptosis in HIE

Apoptosis, the process of programmed cell death, is a progression of well-coordinated and stringently controlled processes prompting cell blebbing, shrinkage, nuclear fragmentation, chromatin condensation, proteolysis, and chromosomal DNA fragmentation [44]. Apoptosis happens in a wide variety of physiological and obsessive situation [45,46]. As of late, ROS have turned into a target for drug discovery since their production is characteristic for the early phases of apoptosis going before the collapse of the mitochondrial membrane potential, release of apoptotic factors, and activation of caspases [47]. Oxidative stress mediated apoptosis in HIE can happen through extrinsic and characteristic pathways. In extracellular apoptotic signaling, inflammatory markers (like TNF-α, TRAIL, Apo3L, and Fas ligand (Fas-L)) respond to HIE, actuate NF-κB, bind to their receptors, lead to caspase-8 activation and the cleavage of the pro apoptotic Bcl-2 family protein Bid to t-Bid, lastly prompt both apoptosis and cell survival triggering intrinsic signs. Oxidative stress mediated mitochondrial injury seems, by all accounts, to be fundamentally engaged with the intracellular apoptotic signaling. The first mechanism is mediated by intermembrane space proteins, for example, CytC, apoptosis inducing factor (AIF), Endo G and Smac/DIABLO (second mitochondria-derived activator of caspase/direct IAP binding protein with a low pI), released into the cytosol. The second mechanism is mediated by proteins of the Bcl-2 family acting directly on the external mitochondrial membrane [48,49,50,51].

3.4. Oxidative Stress Mediated Autophagy in HIE

Autophagy, or type-II programmed cell death, is a conserved, intracellular, lysosome-dependant degradation process that recycle defective proteins or damaged organelles. Autophagy is up-controlled because of oxidative stress, assisting with re-establishing intracellular homeostasis by disposing a number harmful molecules, for example, misfolded proteins overflowing from endoplasmic reticulum (ER) stress, cytosolic proteins injured by ROS, or even dysfunctional mitochondria or ER from prolonged oxidative stress [48,49,50,51]. The autophagy pathway can induce IKKα/NF-κB/I-κB kinase β-mediated proinflammatory signaling via the oxidative stress pathway [52,53]. In parallel, when homeostasis of ROS is disrupted, excessive ROS are gathered in the mitochondria and cytoplasm and can make oxidative damage cells and prompt autophagy [54]. ROS-mediated molecular networks, like PI3K-Akt-mTOR, TLR-4, IGF, MAPK, and AMPK, rely upon a several distinct mechanisms including catalase or caspase activation of autophagy-related genes, and disturbances in the mitochondrial electron transport chain [55]. In any case, oxidative stress mediated autophagy in HIE stays dubious, and whether there is a beneficial or detrimental role of autophagy relies upon whether intracellular stresses have been resolved [56].

II. Clinical Guidelines

1. Imaging the encephalopthic infant:

Neuroimaging is significant in determining the etiology of neonatal encephalopathy, guiding clinical decision making, giving prognosis after hypoxic ischaemic injury and informing risk management and medico-legal proceedings [56].

1.1 Cranial Ultrasound Scanning (CUS)

Standard: All infants with suspected neonatal encephalopathy ought to have a cranial ultrasound scan on admission and ideally before transfer to a regional cooling centre. Standard: CUS ought to be performed, including evaluation of the resistance index, on admission, D1, D4 (post rewarming) and later if necessary (depending upon the MRI). Cranial ultrasound (CUS) stays the most used mode of imaging in these infants offering the advantage of bedside imaging; however, it is examiner dependant and there is poor inter-observer agreement. Cranial ultrasonography is important in identifying different causes for neonatal encephalopathy, for example, congenital anomalies as well as recognizing cerebral haemorrhages and antenatal brain injury. In HIE normal cranial ultrasound findings can be reassuring whereas anomalies in the thalamus and basal ganglia have been associated with adverse outcome [57]. Notwithstanding, predictive accuracy is poor, (sensitivity 0.76 95%CI 0.3-0.97, specificity 0.55 95% CI 0.39-0.7) [58,59]. The combination of abnormal cranial ultrasound and neurological assessment might further improve prediction of neurological results [60]. There is no data suggesting that hypothermia changes the interpretation of cranial ultrasonography [61-63].

1.2 Doppler Studies

Pourcelot's Resistive index (RI) is determined as peak systolic velocity minus end diastolic velocity divided by peak systolic speed. Normal RI of >0.6 is reassuring. Low RI of <0.55 is associated with adverse neurodevelopmental outcome despite the specificity varies [61-63]. The positive predictive value of the resistance index was just 60% (95% CI 45-74%) in infants treated with hypothermia for HIE, considerably less than reported in infants [64]. The negative predictive value of the cerebral resistance index in the cooled infants was 78% (95% CI 6786%) like that reported in non-cooled infants with HIE [64]. While scanning, it is vital to remember that high diastolic flow related with resistive index <0.55 is rarely seen before 6 hours of age [65].

1.3 Possible CUS Findings in HIE

- Early cerebral oedema – generalized increase in echogenicity, indistinct sulci and narrow ventricles.
- Intracranial bleed (e.g., IVH, subdural or extradural hematoma)
- Cortical highlighting
- Following 2-3 days of age, increased echogenicity of thalami and parenchymal echo densities.
- After day 7 cystic degeneration of the white matter
- Increased echogenicity in the white matter seen on day of birth suggest antenatal onset of neonatal encephalopathy **[66]**

2. Magnetic Resonance (MR) Imaging

MR imaging has been displayed to have greater diagnostic and prognostic accuracy than grey scale ultrasonography and is presently considered the imaging modality of choice in neonatal encephalopathy (NE) [56, 67]. CT imaging ought to be limited to emergency situations where there is proof of birth trauma and urgent imaging is required on the grounds that acute neurosurgical intervention is being considered. However, successfully obtaining and interpreting images requires careful preparation and planning. MRI is the imaging modality of choice for diagnostic imaging in NE.

2.1 Preparation

MR imaging of a sick neonate can be difficult and requires careful preparation to get optimal images enabling accurate interpretation. There are a number of safety issues need to be carefully considered.

2.1.1 Timing

The very real anxiety and need for early data about long term prognosis should be tempered by guaranteeing that as much data as possible is obtained from imaging. Injury patterns advance over the first couple weeks and accordingly it is vital to be familiar with the temporal evolution of injury patterns and to consider this in the interpretation of the findings on MRI. In neonates with HIE, specific patterns of injury on conventional MR imaging have been identified as being associated with long term neurodevelopmental problems [68-71]. Ideal timing for a MR examination is somewhere in the range of 5 and 14 days. Before this time, conventional imaging might be generally normal [72, 73]. Also, the infant is often additional stable after the first few days from delivery and is better ready to endure being transported to the MR scanner and the scanning procedure. Assuming

imaging during the first week diffusion weighted imaging (DWI) is essential however may underestimate the extent of the injury, especially in the basal ganglia and thalami [71, 73-75]. Besides, in examples of wide spread injury, and no normal appearing tissue for comparison, it is essential to measure the regional apparent diffusion coefficient (ADC) on the diffusion ADC map. DWI normalizes before the end of the second week.

In a minority of infants early MR imaging (within first the week) might be clinically indicated, either to clarify the diagnosis and exclude other pathologies (for example intracranial haemorrhage, perinatal stroke, metabolic conditions) or in infants where withdrawal of intensive care is being considered. The withdrawal of life sustaining treatment ought not be delayed while MRI is looked for if criteria for discontinuing intensive care, as described in RCPCH and GMC guidance, are met. Sensitivities and specificities for different MR imaging sequences in the first week after birth is shown in Table (1).

Table (1) MR imaging sequences in the first week after birth

Imaging Test	No of studies	No of patients	Pooled sensitivity		Pooled specificity	
			Point estimate	95% CI	Point estimate	95% CI
MRI DWI first week	2	36	0.58	0.24-0.84	0.89	0.62-0.82
ADC first week	3	113	0.79	0.5-0.93	0.85	0.75-0.91
T1/T2 first week	3	60	0.84	0.27-0.99	0.9	0.31-0.99
T1/T2 first 2 weeks	3	75	0.98	0.8-1.0	0.76	0.36-0.94
MRS first week	3	66	0.75	0.24-0.96	0.58	0.23-0.87
MRS first 2 weeks	3	56	0.73	0.3-0.97	0.84	0.27-0.99

2.1.2 Requesting MR Image

It is important to give clear and concise clinical details to the radiology team not only to facilitate interpretation of the scans in the light of the clinical history yet in addition to guarantee that the department are aware of the current clinical status of the infant and can prepare for the scan properly.

2.1.3 Sedation

Imaging the neonatal brain depends on the infant being still. Neonates might be imaged during natural sleep following a feed. Wrapping up can help this ('feed and wrap' technique). Be that as it may, the quality of MR images is frequently compromised by movement artefact, consequently lessening the detailing accuracy and the ability to anticipate neurodevelopmental outcome. In non-ventilated infants, light sedation can be accomplished with chloral hydrate empowering better quality pictures. With strict protocols and adequate monitoring, chloral hydrate sedation for MR filtering can be safely performed for both preterm and term infants [76,77]. Chloral hydrate 30-50 mg/kg ought to be managed by means of oral or nasogastric route on an empty stomach (1 hr fast) around 15 minutes before the expected beginning of the scan. The rectal route might be utilized if oral/nasogastric administration is not possible. The chosen dose ought to be judged through cautious clinical assessment and changed appropriately relying upon concomitant administration of narcotics and anticonvulsants. Sedation might bring about hypoventilation and the requirement for supplemental oxygen albeit the occurrence of critical complications was 1% [76]. Subsequently, oxygen saturation is observed continuously from time of sedation to time of full waking and neonatal-trained staff should be available all through. infants who are already appropriately sedated for ventilation do not routinely need any extra sedation.

2.1.4 Monitoring

All infants, sedated or not, ought to be observed during transportation to and from the scanner as well during the actual technique. MR compatible pulse oximeters are accessible in all MR departments for this reason. Electrocardiogram monitoring ought to likewise be undertaken during transportation and during the scan where suitable MR compatible equipment is accessible. Two neonatal qualified staff ought to be in attendance all through the scan for all ventilated infants. The help of a pediatric anaesthetist can likewise be useful. No less than one neonatal/pediatric nurse ought to be in participation for all patients requiring sedation. Observations ought to be documented routinely during both transport and scanning.

2.1.5 Equipment

Transferring sick infants to the MR department is challenging. It requires careful planning and an understanding of the potential risks implied. It is in this manner crucial that staff going with the infant should be familiar about all the equipment (for example transport incubator, infusion pumps, MR compatible equipment) and be skilled in the stabilization of a sick neonate. Notwithstanding MR compatible monitoring equipment, ventilated infants will require a MR compatible ventilator. Such infants frequently have multiple infusions. Before leaving the neonatal unit, check stability and that baby is metal free with all appropriate lines secure. A metal check of the baby (for example for arterial lines with terminal electrode, poppers on clothes, electronic IDs and so on) and of all staff needs to consideration prior to going into the MRI scanner room. Cautious thought likewise should be given to the method to be continued in case of clinical deterioration of the infant during the scan. Just MR compatible resuscitation equipment can be taken into the scanner room. In the event that this isn't accessible, the infant should be brought out scanner room before resuscitation and stabilization. It is critical that all individuals of staff know about the resuscitation method during transportation and scanning.

3. MR Details

Neonates present explicit difficulties to the items of common sense of obtaining a scan in view of their size and the expanded water content of their developing brain.

3.1 MR Coil

A standard adult head coil ought to produce an adequately high signal to noise ratio. In a large coil care should be assumed to position the neonatal head in the centre of the loop (padding under the head), to keep away from uneven signal intensity in the acquired images. High signal to noise and, even signal intensity might be gained utilizing a smaller coil, for example, adult knee coil or a dedicated neonatal head coil. Poor coil choice or head position can bring about low quality images.

3.2 MR Sequences

Table 2 MR Sequences

Essential MR Sequence		**Recommended MR sequence**	
Axial T1	To visualise basal ganglia & thalami & for assessing myelination in the posterior limb of internal capsule.	**Fluid attenuated inversion recovery**	Useful for detecting late gliotic changes in older infant.
Sagittal T1	Ideal for visualising midline structures (e.g. pituitary, corpus callosum, cerebellar vermis).	**Venogram**	Exclude sinus thrombosis & differentiate from subdural haemorrhage.
Axial & coronal T2	Ideal for identifying early ischaemic changes. and for assessing grey-white matter differentiation. Detection of haemorrhage.	**Angiogram to include proximal cerebral arteries and neck vessels**	Visualise cerebral vessels in focal stroke and exclude carotid dissection.
Gradient Echo Axial	Greatest sensitivity for detecting intracranial haemorrhages.	**MR spectroscopy**	Deep grey matter Lac/ Naa ratios have demonstrated greatest prognostic sensitivity (18) detection of elevated lactate or glycine in certain metabolic disorders.
Diffusion Weighted image	Detects ischaemic changes earlier than conventional MRI. Particularly useful if focal stroke suspected	**Motion resistant Sequences**	Propeller/ BLADE or T2 single shot FSE.

4. Reporting

To give an informed and precise assessment regarding a MRI scan relating the image with the clinical history and current findings of the patient. MRI scans ought to be reported for by properly experienced personnel and inspected inside the setting of MDT/clinic-radiological meetings. It could be workable for a MRI scan to be acted in a local centre however there may not be proper expertise to report the images. Plans might be made for tertiary reporting in these cases. Images ought to be moved to tertiary radiologists utilizing proper NHS routes (e.g. PACS).

A standardized revealing scheme guarantees all areas are reviewed, promotes accessibility of results, works with interpretation of subsequent imaging and helps in auditing the outcomes. Clear interaction for communication between the referrer and reporter ought to be accessible so an appropriate clinically based assessment of imaging can be given and communicated to family.

5. Serial Imaging

The exactness of early, properly planned MR imaging in predicting neurodevelopmental outcome is documented and negates the requirement for routine serial scans in most of infants [3]. Notwithstanding, it very well might be appropriate to repeat MR scan where the initial scans has been undertaken within the first week of life when MR changes are as yet advancing or when shown by the clinical course of the infant, or in the case of significant movement artefact on previous scan precluding satisfactory interpretation. Ensuing imaging ought to be undertaken at the discretion of the clinician responsible for the infant in conversation with consultant radiologists.

6. Features of HIE in MR imaging

Following moderate or severe HIE, especially following a recorded sentinel event, abnormal signal intensity is most commonly distinguished in the basal ganglia and thalami, corticospinal tracts, the subcortical white matter, and regional cortex [70] and images have high predictive values for recognizing adverse results. Extensive and dominant white matter and cortical injury is suggestive of additional chronic hypoxic ischaemic compromise as might be shown by fetal growth restriction (FGR) and additionally poor fetal movements. It might likewise complicate symptomatic hypoglycaemia and/or bacterial or viral infection for example Parecho infection. On MR spectroscopy high lactate (suggestive of tissue hypoxia and ischaemia) and low N-acetyl aspartate (reflects neuronal injury) within the basal ganglia and thalami is frequently seen. The predictive precision of MRI is unchanged following therapeutic hypothermia [78,79].

7. Communication with Parents

This is an exceptionally stressful time for parents and the uncertainty about long term prognosis adds to this. Timely and repeated communication with parents and family ought to be a vital role of really focusing on these sick infants. Concerning imaging, it is vital to talk about in advance what data might be acquired by imaging the infants at that specific time and the limitations of the imaging modality. While MR imaging can give dependable prognostic indicators, it is vital to likewise consider all neurological assessment tools and information while discussing long term prognosis (including clinical examination, course, resistive index on CUS and aEEG/conventional EEG findings). Focusing on the requirement for long term developmental follow up and support for these infants. It is imperative that results of imaging are communicated to parents at the earliest opportunity by the most senior clinician available, should be the consultant responsible for the infant. This should ideally be undertaken face to face. Neuroimaging is a significant part of neuro-intensive care of infants with HIE. It can give vital data to guide management and prognosis of these infants.

8. Audit Standards

1. Infants with neonatal encephalopathy ought to go through MRI Ideally sedation ought to be utilized.
2. Optimal timing for MR imaging in cases of HIE is between 5-14 days after birth.
3. Standardised reporting by a radiologist with suitable experience.
4. Documentation of monitoring during MR imaging.
5. Adverse events related to sedation and MR imaging.

10. THE SARNAT CLINICAL STAGES OF HIE

In 1976, Sarnat and Sarnat were dealing with developing a clinical ranking system to assist with working on the diagnosis of hypoxic-ischemic encephalopathy. HIE is a serious type of birth asphyxia (or oxygen deprivation) that can cause brain damage, disability, and death. Children with HIE are at higher risk for other permanent conditions, including Cerebral Palsy.

They proposed a three stage system for classifying HIE.

Stage I: describes conditions of mild HIE.

Stage II: describes conditions of mild to severe HIE.

Stage III: describes conditions of severe HIE.

STAGE I

In Stage I, or mild cases of HIE, the infant's right symptoms just after birth include:

- Hyper alertness from the infant
- slightly decreased muscle tone (floppy muscles)
- Brisk deep ligament reflexes (e.g. a knee-jerk reaction)
- Fussiness

- Difficulty feeding
- Trouble sleeping
- Frequent crying

Frequently, these symptoms will disappear within less than 24 hours from the child being born. In any case, all symptoms of HIE ought to be carefully monitored and treated properly.

STAGE II

In Stage II, or moderate to extreme HIE, the infant might show symptoms of:

- Unusual lethargy
- Significant hypotonia
- Lower deep tendon reflexes (less response to reflex stimulus)
- Difficulty grasping with the hands
- Moro Reflex (the sensation of suddenly falling, certain individuals experience this right as they fall asleep a baby will respond to this sensation by shooting

  their arms out and may try even gasp)
- Disinterest in sucking
- Trouble breathing, or apnea (a momentary cessation of breathing)
- Seizures

In moderate to severe HIE, it is vital that the child is appropriately observed and treated. Effective medical care may significantly decrease the child's prognosis. The first several weeks are the most critical period for observing and treating a child with Stage II HIE.Many of the causes for HIE are no doubt preventable with careful monitoring and quick treatment. It's a sad reality that medical mistakes and doctor carelessness are common causes of HIE.

STAGE III

In Stage III, or severe cases of HIE, the symptoms might include:

- An unresponsive, coma like stupor
- No response to physical stimulus
- Extreme difficulty breathing
- Generalized hypotonia (floppy muscles over the whole body)
- Depressed deep tendon reflexes
- No neonatal reflexes (sucking, swallowing, grasping, Moro)
- Vision problems
- Dilated, fixed, or unresponsive pupils
- Delayed seizures (expanding following 24 - 48 hours, resistant to treatment)
- Irregular heart beat
- Poor blood pressure

Once more, proper monitoring and treatment of Stage I severe cases of HIE is fundamental to improving the child's condition. Severe cases of HIE may cause super durable injury or even death. It is the clinical professionals' obligation to carefully watch the baby's symptoms as they develop and to respond to symptoms of HIE with an effective treatment plan. In the event that a clinical

professional notices symptom of HIE, they might wish to arrange testing, including utilizing a magnetic resonance imaging machine (or MRI) to examine the child for damaged brain cells. Intervention within early time following birth is critical. Getting suitable clinical consideration in a Neonatal Intensive Care Unit (or NICU) may significantly improve the state of the infant, diminish further complications, and limit related injuries. [70]

III. Clinical Strategies in HIE

1.Antioxidants

Throughout the last 10 years, hypothermia has been established as the standard treatment for HIE, and further investigations for a multi-targeted approaches have to be explored in depth. Interventional targets have comprised of pathways associated with inflammation, apoptosis, and autophagy followed by oxidative stress in experimental translational studies.

2. Hypothermia

Hypothermia is routinely utilized as a protective therapeutic tool for moderate to severe HIE in clinical application [80]. Selective head cooling (34.5 °C) and total body cooling (33.5 °C) are the two therapeutic hypothermia modalities. The decrease in temperature influences all physiological systems of the body, including redistribution of blood flow and disturbances of microcirculation, ultimately prompting the decrease of metabolism and oxygen supply to tissues. At the point when hypothermia is utilized in HIE it maintains with or improves the level of antioxidants [81]. Therapeutic hypothermia should start within the initial six hours after birth, and be kept up for three days. Studies have shown that this treatment is successful for diminishing cerebral injury and improves the brain outcome secondary to hypoxic-ischemic attack in full-term-born and near term preterm new-born [82].

3. Erythropoietin (EPO)

There is convincing preclinical research and clinical proof that(EPO) can promote the expression of anti- apoptotic genes comparative to apoptotic genes, inhibit inflammation, attenuate oxygen free radicals, decline caspase activation, and increment neurogenesis in light of HIE through the cross talk between PI3K/AKT, STAT5, and ERK molecular signal pathway [83,84,85]. Right now there are two active clinical preliminaries (Neonatal Erythropoietin and Therapeutic Hypothermia Outcomes in Newborn Brain Injury (NCT01913340) and Efficacy of Erythropoietin to Improve Survival and Neurological Outcome in Hypoxic Ischemic Encephalopathy (NCT01732146)) examining EPO in combination with hypothermia in infants with HIE. The NCT01913340 assesses an EPO dose of 1000 U/kg/dose IV × 5 dosages, while the NCT01732146 assesses EPO intravenous infusions (5000 U/0.3 mL) 1000 to 1500 U/kg/dose multiple times given each 24 h with the primary dose within 12 h of delivery [86,87]. Studies have shown that EPO administration is safe in neonatal brain. Combining cooling with EPO in HIE could improve the recovery of sensor motor function, behavioural and cognitive responses, and histological integrity, improve motor responses and cognitive responses, promote cerebellar growth, and lessen death or disability [88,89,90].

4. N-Acetyl-5-methoxytryptamine (Melatonin)

Melatonin, a strong endogenous indolamine, plays shown a neuroprotective role. Melatonin, has anti-inflammatory, anti-oxidant, and anti-apoptotic properties in HIE. Melatonin′s protective actions are believed to originate from the interactions of its receptors (receptor dependant actions), its direct free radicals scavengering (receptor-independent actions), and on account of yet-undefined actions. Melatonin openly crosses the placenta and blood-brain barrier making it more attractive [91,92,93]. A previous study has shown that pre-treated melatonin in asphyxiated term neonates (got hypothermia combined application with melatonin 10 mg/kg × 5 days, oral.) is feasible and may ameliorate brain injury. Almost certainly, higher doses are required to obtain an antioxidant impact and this high dose might even desensitize the melatonin receptors [94].

5. N-Acetyl serotonin (NAS)

NAS has likewise been demonstrated to be preferable as scavenging peroxyl radicals than melatonin itself. The neuroprotective impacts of NAS in HIE may result through its consequences for cells mitochondrial impairment, including permeability transition pore opening, fragmentation, inhibition of the subsequent release of apoptogenic factors from mitochondria into the cytoplasm, activation of apoptosis protein expression, and suppression of the activation of autophagy under oxidative stress conditions [95].

6. Magnesium Sulphate (MgSO4)

A lot of researches demonstrates the way that MgSO4 can diminish secondary inflammation and associated injury that occurs under oxidative stress. The conceivable mechanism might be for the way that MgSO4 can bind to the magnesium site on N-Methyl-D-aspartate (NMDA) glutamate channels, inhibiting free radical production, and stabilizing the cell membrane [96,97,98,99]. There is currently evidence from meta-analysis of randomized controlled preliminary studies that antenatal administration of MgSO4 is related with a small however significant decrease in the risk of cerebral palsy and gross motor dysfunction after preterm birth [100,101].

7. Stem cells

Consolidated treatment with hypothermia and transplantation stem cells (e.g., amnion epithelial cells (AECs), hematopoietic stem (HSC), umbilical cord blood (UCB), UCB-derived endothelial progenitor cells (EPCs), and bone marrow-derived mesenchymal stem cells (MSCs)) has been regarded as a therapeutic strategy to promote functional recovery in animal models of HIE. A study has shown that the collection, preparation, and infusion of fresh autologous UCB cells for use in infants with HIE is feasible [102]. Notwithstanding stem cell transplantation, there is ongoing research in the field of stem cell factors (e.g., G-CSF) [103,104,105,106].

8. Edaravone (3-Methyl-1-phenyl-2-pyrazolin-5-one)

Edaravone is a free radical scavenger that is believed to be valuable in the treatment of post-ischemic neuronal dysfunction, and further developing memory and ability to learn in HIE. Utilizing brain microdialysis, electron paramagnetic resonance (EPR) spectroscopy, photograph acoustic imaging, and laser speckle contrast imaging identification, research on edaravone has shown that it is believed to interact with peroxyl and hydroxyl radicals, lipid peroxidation, and DNA peroxidation, and that it makes a radical intermediate that forms stable oxidation items. clinical studies show, in the treatment group treated with edaravone alone or in mix which other neuroprotective medications (like Ganglioside, hyperbaric oxygen (HBO), Xingnaojing, and so forth) the distinction of national institutes of health stroke scale (NIHSS), proinflammatory, anti-inflammatory cytokines, free radicals were measurably significant [107,108,109,110].

9. Allopurinol

Proof exists that recommend that allopurinol, a xanthine-oxidase inhibitor, functions as a chelator of non-protein bound iron, as well as a direct scavenger of free radicals, proposing that allopurinol might be an assistant to therapeutic hypothermia in HIE [111,112,113,114]. In a recent study, treatment with allopurinol in HIE infants with hypoplastic left heart condition fundamentally impeded the biochemical cascade of neuronal damage, including seizures, death, coma or cardiovascular events, in contrast with a control group [115].

10. Osteopontin (OPN)

Recent studies show that OPN, a multifunctional glycoprotein, is controlled in brain tissue affected by HIE. Exogenous OPN has diminished infarct volume and improved neurological outcomes. OPN-induced neuroprotection was cleaved with cut caspase-3 inhibition, regulation of cerebral cell proliferation, oligodendrocyte differentiation, and anti-apoptotic cell death [116,117,118].

11. Flunarizine

Flunarizine, a particular Ca2+ channel blocker, which has strong neuroprotective properties against hypoxic-ischemic encephalopathy, acts according to mechanism free of consequences for dopamine release [119].

12. Nitric Oxide

Inducible NO synthase (iNOS) is prompted to produce exessive NO in which prompts cascade responses of inflammation and neuronal death in HIE. Nitric oxide, an iNOS inhibitor, through the NF-κB/nNOS pathway plays a neuroprotective role by expanding iron deposition, inhibiting platelet and leukocyte adhesion, keeping up cerebral blood flow, and preventing neuronal injury. NO has been demonstrated to be another therapeutic agent in the treatment of brain hypoxia-ischemia [11,12,120,121].

13. Hydrogen Peroxide (H2O2)

Most viewed studies show that H2O2, either in the gas or fluid form, goes about as a treatment for hypoxia/ischemia through preconditioning protection. Evaluated by 2,3,5-triphenyltetrazoliumchloride (TTC), Nissl and TUNEL staining, and caspase-3 and caspase-12 activities in the cortex and hippocampus, H2O2 might act through theHIF-1α pathway inhibiting neuronal apoptosis and attenuating cerebrovascular reactivity (CR) to hypercapnia, N-Methyl-D-aspartate (NMDA), norepinephrine, and sodium nitroprusside [122,123,124,125].

14. N-Terminal Tripeptide of IGF-1 (GPE) IGF-1 and Insulin-Like Growth Factor-1 (IGF-1) and GPE is a polypeptide chemical that has been researched as a potential neurotrophic factor for the treatment of HIE. IGF-1, by means of the PI3K/Akt/GSK3β and NF-κB pathway phosphorylation, attenuates activation of caspases and mitogenic impacts. Previous research has shown that the neuroprotective activities of IGF-1 infusion were global, robust, and showed a wide compelling rose range and treatment window [126].

15. Barricade of Connexion Hemi Channels (Connexons)

Expanding proof supports that suppressing the induction or action of the connexion proteins shaping hemi channels contributes to HIE. Previous research has uncovered that unopposed connexons additionally assume a significant role. They mediate the release of paracrine molecules, which thusly send cell death messages by the secretion of intracellular mediators, (for example, ATP, Nicotinamide adenine dinucleotide (NAD+), and glutamate), which eventually prompts cell edema [127,128].

16. Naloxone and β-FNA

Naloxone, a μ-narcotic receptor blocker, and β-FNA, a μ-narcotic receptor antagonist, both attenuate myeloperoxidase action and chemokine (macrophage inflammatory protein-1 alpha and - 2) mRNA expression through C-fos, C-jun, Nur77, and the MAPK pathway in HIE [129].

17. Salvia

Studies have shown that Endothelin-1 (ET-1), NO, and CK-BBin in both the blood and cerebrospinal fluid (CSF) took part in the pathological process and determine the therapeutic effects of HIE. Salvia injection exhibited a therapeutic effect better than a control group at a statistically significant level [130].

IV. Conclusion

HIE is one of the main causes of morbidity and mortality in neonates. In this review, we featured the molecular mechanism and protective strategies for oxidative stress in HIE, predominantly focusing on mechanisms related to anti-inflammatory, anti-apoptosis, and regulation of autophagy. Therapeutic interventions empowering the avoidance or decrease in hypoxia-induced brain damage before or during an early stage of free radical production will require proceeded with investigations to decide optimal effectiveness. In addition, the safety and efficacy of these combinatorial strategies for HIE can be maximized by following appropriate translational research guidelines.

V. Abbreviations

EEG : Electroencephalography

HIE : Hypoxic-ischemic encephalopathy

ROS : Reactive oxygen species

SOD : Superoxide dismutase

VI. Conflict Of Interest

All authors declare no conflicts of interest.

VII. AUTHORS CONTRIBUTION

Authors have equally participated and shared every item of the work.

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