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Background

Neonatal hypoxic-ischemic encephalopathy (HIE) is a serious brain injury resulting from severe reduction of cerebral blood flow and oxygen around the time of birth. Affected neonates can display altered levels of consciousness, decreased or absent reflexes and muscle tone, seizures and signs of organ failure. Accounting for 1 million deaths a year which is 24% of all neonatal deaths worldwide (See Figure 1), neonatal HIE is a primary global medical concern. It occurs in 1-8 cases per 1,000 live births in developed countries, and in as many as 26 per 1,000 live births in developing countries. Around 25-50% of neonates with HIE will die within the first two weeks and around 1/3 will survive with brain damage. Untreated moderate to severe HIE leads to 60-65% risk of cerebral palsy, mental disorders, visual and hearing impairment, epilepsy and learning disabilities. As such, HIE carries a significant health and economic burden on the family and community.

These include maternal factors like cardiovascular collapse; uterine factors such as uterine rupture, premature separation of the placenta (placental abruption) and fetal factors, such as cord prolapse, tight cord coil and shoulder dystocia. However, only 15-29% of patients with HIE have a documented sentinel event. Examination of the placenta may provide valuable information about the timing and the cause of the HI, such as placental infarcts, immaturity, small size and weight which compromise placental perfusion. Still, a significant number of HIE has no identifiable risk factors, making the duration and timing of insult impossible to determine. The general term neonatal encephalopathy (NE) is used when there is no clearly identifiable acute hypoxic-ischemic event peri-partum. NE is a heterogeneous condition which may result from prematurity, genetic, infectious and other causes aside from hypoxic-ischemic (HI) injury.

It is imperative to be aware of the mechanisms of hypoxic-ischemic insult in neonates to understand the timing and mechanism of action of emerging therapies such as therapeutic hypothermia. The significant reduction in placental blood flow to the fetus will lead to a fall in the fetal cardiac output. The fetus compensates initially by redirecting blood flow to 3 vital organs—the brain, heart and the adrenal glands. However, prolonged or severe oxygen deprivation to the brain exhausts these mechanisms and triggers a cascade of cellular processes, leading to inflammation and cell death.

In cases of moderate reduction in the cerebral blood flow (CBF), there is an opportunity to redirect the cerebral arterial blood flow from the anterior circulation to the posterior circulation to protect the perfusion in the critical posterior structures, such as the brain stem, cerebellum and the basal ganglia. In this instance, the brain injury will be limited to the cerebral cortex and to the watershed areas of the cerebral hemispheres. However, if the oxygen deprivation is abrupt and intense, there is no chance for redirection of the blood meaning that the basal ganglia and the thalami become injured - a characteristic of HIE.

Pathophysiological Features of Hypoxic Ischemic Encephalopathy

Brain injury in HIE evolves over hours, days and even months. It follows a temporal sequence of brain injury which can be divided into 3 phases (See Figure 2).
NEONATAL HYPOXIC-ISCHEMIC ENCEPHALOPATHY PATHOPHYSIOLOGY FEATURES

Figure 2: Schematic Overview of the Pathophysiological Features of Hypoxic-ischemic Encephalopathy


PRIMARY ENERGY FAILURE

The first phase is called the primary energy failure which occurs 0-6 hours after the hypoxic-ischemic injury. With insufficient blood supply in the placenta, hypoxic-ischemic injury occurs to fetal tissues, which compromises cardiac contractility. This leads to systemic hypotension and decreased cerebral blood flow. The oxygen and glucose supply to the brain is compromised, triggering an alternative energy pathway—the anaerobic metabolism. This is an inefficient pathway resulting in lower energy (ATP) production, and an increase in lactic acid accumulation. The result is a concomitant nerve membrane depolarization with an influx of calcium inside the cells. Through the calcium transporters, there is a pronounced release of excitatory amino acids (EAA), like glutamate, into the extracellular space. Buffering and cellular reuptake of these EAA require energy, but, due to energy depletion, high EEA levels accumulate, leading to glutamate neurotoxicity in particular. There is also a release of enzymes (peroxidases), which breaks down the neuronal membrane. The combination of lactic acidosis, glutamate release, lipid peroxidation and toxic effects of the excitatory amino acids and nitrogen species lead to cell death called necrosis.

LATENT PHASE

A partial recovery follows 30-60 minutes after the primary energy failure. This ushers the latent phase of injury, which lasts from 1 to 6 hours- characterized by recovery of the oxidative metabolism of the mitochondria, but with continuation of inflammation and the apoptotic cascade. Neonates with mild HIE will recover at this phase, but those with moderate to severe HI injury will continue on to the second phase. The latent phase is a window during which treatment options like therapeutic hypothermia may be initiated to prevent progression of injury to the second phase.

SECONDARY ENERGY FAILURE

A secondary energy failure phase occurs 7 to 72 hours after the hypoxic-ischemic insult. There is a reperfusion in the brain, accompanied by a burst of excitatory transmitters and free oxygen radicals. Mitochondrial dysfunction becomes more serious and the ATP reserves are critically depleted. As the phase progresses, the mitochondria release the Cytochrome C and the injury cascade is reactivated and nerve cell apoptosis begins. Inflammatory factors like cytokines are released and the brain injury becomes more substantial. Seizures are often apparent during this phase.

TERTIARY ENERGY FAILURE

Phase 3 occurs 72 hours from onset of the HI injury and may last for days to months. Depending on the severity of the HIE and response to the therapeutic interventions, there are two possible outcomes. One involves recovery where the brain tissues enter a repair process and the surviving nerves and glial cells begin to differentiate, proliferate and regenerate. However, if the insult is severe, the injured tissues continue to deteriorate with persistence of the inflammation. The supporting cells like the glia and the astrocytes continue to release harmful cytokines, leading to additional neuronal deaths, despite restoration of oxygen and blood supply to the brain.

Mechanisms of Injury in Hypoxic Ischemic Encephalopathy

EXCITOTOXICITY

Under normal conditions, the excitatory amino acids, like glutamate, play a crucial role in fetal brain development, such as synapse formation, neural plasticity, memory and cognition. However, with energy depletion during the hypoxic-ischemic insult, the nerve and its supporting cells such as the glial cells will depolarize and release a large amount of glutamate into the extracellular cell, reaching neurotoxic levels. There is also intracellular calcium elevation, which triggers enzymes, mitochondrial dysfunction and over production of reactive oxygen radicals and nitrogen species. Furthermore,
Depolarization of the nerve membrane leads to an influx of sodium and chloride, followed by water, resulting in edema and rupture of the cell. These may lead to clinical seizures.

**OXIDATIVE STRESS**
Free radicals are highly reactive molecules with unpaired electrons. These are crucial in cell signaling and regulation of smooth muscle tone, cerebral blood flow regulation, inflammation and modulation of the excitatory transmission. However, in hypoxic and ischemic insult, they participate in degenerative processes leading to cell death. The most important source of radicals is the oxidative metabolism in the mitochondria. The radicals like superoxide anions are produced a few minutes or hours after the insult. The radicals cause changes in the cellular components leading to cell death.

**INFLAMMATION**
Hypoxic-ischemic injury initiates massive inflammation over a few hours. Again, the supporting cells, like the glia and the astrocytes, activate immuno-active molecules like the cytokines, growth factors and chemo-attractants, which lead to inflammation.

**BLOOD BRAIN BARRIER PERMEABILITY**
Cerebral vessels are fully supported by astrocytic processes, which aid in forming a semi-permeable blood brain barrier. In cases of brain insults like stroke, there is an increase in blood brain permeability, allowing extravasation of plasma constituents and the development of brain edema. Breakdown of the BBB may trigger clinical seizures.

**Severity of the HIE**
Neonates suspected with HIE are classified according to the Modified Sarnat and Sarnat Staging (Figure 3). The scoring system is divided into stage 1- mild, stage 2- moderate and stage 3- severe. The neonate’s status may progress over hours to days depending on the severity of the insult. Mild HI has the best prognosis with almost 100% recovery. The infant is hyper-alert, with increased tone and reflexes. Stage 2 is characterized by the presence of seizures, some decrease in responsiveness and abnormal muscle tone. Seventy-five percent will fully recover, but 25% of the neonates, especially those who are still symptomatic after 1 week, have a higher risk of long-term neurological impairment. In cases of severe HIE (stage 3), the infant is already in a coma, displaying no respiratory effort with absent or decreased reflexes and muscle tone. All of them will either die or have severe neurologic disabilities.

<table>
<thead>
<tr>
<th>Level of consciousness</th>
<th>Mild HIE (I)</th>
<th>Moderate HIE (II)</th>
<th>Severe HIE (III)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle tone</td>
<td>Mild hypotonia</td>
<td>Lethargic</td>
<td>Stuporose</td>
</tr>
<tr>
<td>Complex reflexes</td>
<td>Flaccid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suck</td>
<td>Normal/Weak</td>
<td>Weak/Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Moro</td>
<td>Strong</td>
<td>Weak/Incomplete</td>
<td>Absent</td>
</tr>
<tr>
<td>Seizures</td>
<td>Absent</td>
<td>Common</td>
<td>Frequent/difficult to control</td>
</tr>
</tbody>
</table>

Figure 3: Modified Sarnat Staging of Hypoxic Ischemic Encephalopathy

*Source: Neonatal Intensive Care Unit Clinical Guideline. Ashford and St Peter’s Hospital. Dr. Peter Reynolds. June 2013.*

**Assessment of Hypoxic Ischemic Encephalopathy**
The rapid assessment of neonates at risk for HIE is important, since therapeutic strategies may need to be initiated early on to optimise the outcome. Unfortunately, in clinical practice, suspicion for HIE is based mainly on the overall clinical picture, which is often non-specific. Furthermore, an acute hypoxic-ischemic insult in close temporal proximity to labor and delivery should be present; but oftentimes, may be challenging to identify. Still, based on the AAP and the ACOG guidelines for HIE, all of the following conditions should be present for the infant to be considered as having HIE, or what was previously referred to as perinatal asphyxia:

- Profound metabolic or mixed acidemia (pH<7.0 or base deficit > or = 12 mmol/L) in the umbilical arterial sample
- Persistence of an Apgar score of 0-3 for longer than 5 minutes
- Neonatal neurologic dysfunction (altered sensorium, seizures, absent or decreased tone or reflexes)
- Presence of multi-organ failure (kidney, liver, lung, heart and intestines)

Contributory factors consistent with an acute peripartum HIE include:

- Sentinel hypoxic or ischemic event occurring immediately before or during labor or delivery such as uterine rupture or severe abruption placenta
- Fetal heart rate monitor patterns consistent with acute peripartum event (category III pattern)
- Neuro-imaging revealing brain injury typical of HIE
- No evidence of other proximal or distal factors that could contribute to an HIE

Neuro-imaging
Once HIE is suspected, neuro-imaging can identify HIE injury pattern as early as the first 48 hours. However, infants are most often unstable, so the neuro-imaging cannot take place. Also, neuro-imaging techniques like head ultrasound and MRI may not show the presence or the exact extent of injury early on since the injury may still be evolving. In the majority of HIE patients, therapy is started based on the clinical picture without the aid of neuro-imaging. Neuro-imaging is still essential, not only for identification of the timing and characteristic of the HIE injury, but more importantly, for prognostication.

Cranial ultrasound may be a useful bedside tool to determine the presence of hemorrhage or ventricular size. However, it has poor prognostic capability and may only visualize increased echogenicity of the basal ganglia and thalamus in severe HIE. Ultrasonography is therefore, not the standard neuro-imaging tool for HIE. Head CT Scan is also rarely used due to exposure of the neonate to the high radiation dose which is used to achieve adequate resolution of the brain parenchyma.

Magnetic Resonance Imaging (MRI) is the preferred imaging tool to determine the extent and severity of brain injury and prognosis. Imaging is performed preferably on the 5th to the 14th day of life, after therapeutic cooling and rewarming have been completed. In moderate to severe HIE, abnormal signal intensities in the basal ganglia and thalami, corticospinal tracts, white matter and cortex are seen in the classic MRI image. For infants with acute hypoxic-ischemic insults, the pattern of injury is predominantly in the basal ganglia and the thalamus. In the first 7 days, low diffusion coefficient values in the basal ganglia can already predict adverse neurologic outcomes. Diffuse basal ganglia and posterior limb of the internal capsule injuries are associated with hearing and visual impairment, severe CP and death. Nevertheless, there should be a prudent interpretation of a normal MRI performed after therapeutic hypothermia, since the neurologic outcome can differ. Magnetic Resonance Spectroscopy (MRS) allows an in vivo quantification of the brain metabolites and may serve as an early marker for brain injury. While MRI may still be normal in the first 24 hours, MRS already shows abnormalities. It is becoming used increasingly for both clinical and research prognostication. An elevated lactate/N-acetyl-aspartate ratio has high sensitivity (82%) and specificity (95%) for long term neurologic impairment.

Neurophysiology
EEG and aEEg are important tools to assess severity and prognosis of HIE.

The aEEG is a bedside test which can monitor the neonate for >24 hours. With therapeutic hypothermia, the optimal time to assess aEEG for prognosis is at 48 hours after the insult. Still, it can only detect 1/3 of single seizures and 2/3 of repeated seizures. Shorter seizures lasting for less than 30 seconds are often missed.

The multi-channel EEG is the gold standard for neurophysiologic tests, but bedside availability and delays in interpretation pose a real problem. Abnormalities in the background EEG pattern and the loss of the sleep-wake cycling are commonly seen early after the HI injury. Normal to mildly abnormal EEG (e.g. mild excess in discontinuity), or early recovery from severe abnormalities within the first 24-36 hours after birth signifies a good prognosis. Whereas severe abnormalities (e.g. burst suppression, depressed and undifferentiated tracing, extremely low voltage) are associated with moderate to severe brain injury, death or disability.
Biomarkers

Monitoring, evaluation of treatment response and prognostication may be measured with a combination of neurologic examination, MRI and EEG. However, the infant is often too sick to be transported to the MRI unit and hypothermia may depress the aEEG tracings; limiting their predictive capabilities. Biomarkers may potentially prognosticate earlier and as such, enable the physician and the family to decide on treatment options. Some can be detected in the peripheral blood smear while others are only detected in the CSF due to limited BBB permeability. Candidate biomarkers include the neuro specific enolase (NSE) released from damaged brain cells which shows elevated levels in the first 4 to 48 hours of life in infants with HIE. While the myelin basic protein, a major protein of the myelin, rapidly increases after the HI insults and correlates well with white matter damage. Acidic calcium binding protein S1000βis released by the injured astrocytes and dramatically rises within the first hour after the HI injury. Elevated malondialdehyde (MDA) levels imply mitochondrial oxidative stress. Biomarkers of neuroinflammation such as interleukin, TNF alpha, high sensitivity CRP are also seen in neuronal damage, but their induction is delayed. Further studies are needed before the biomarkers become an integral part of the diagnosis and prognostication in HIE.
REFERENCE: