Post Cardiac Arrest CPR Related Brain Death: Pathology, Clinical Manifestations and Diagnosis

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CEREBRAL ANOXIA AND HYPOXIC BRAIN INJURY

Cerebral anoxia is a broad term that is associated with deterioration of the brain in the event of decreased or interrupted blood flow to the brain tissue. The term Ischemic anoxia is used when the vascular supply of the brain is interrupted such as after a cardiac arrest [1]. Anoxic anoxia occurs due to the lack of oxygen in the lungs such as strangulation, near drowning or effects of certain anesthetic agents. Other types of anoxia include anemic anoxia and toxic anoxia which are associated with critically decreased hemoglobin levels and buildup of toxic metabolites, respectively [2].

Literature shows that the mortality rate after ischemic anoxia has been decreasing over the years with improving survival rate. This may be due to the improvement in emergency care

and advancement in intensive care unit with greater number of patients undergoing cardiac rehabilitation [3]. There is extensive literature on the clinical manifestations of focal brain lesions but very few studies focus on the effects cerebral anoxia and its consequences on cognition and behavior. Before assessing the specific manifestations of anoxia, it is critical to distinguish between the different types of anoxia [4,5].

In anoxic anoxia, since it is mainly the lack of oxygen while other nutrients such as glucose are able to reach the brain tissue, it is reported that these patients perform significantly well neuro-psychologically when compared to patients with ischemic anoxia with complete interruption in oxygen and nutrient supply to the brain [5]. Similarly, another study suggests that different type of anoxia then may produce different cerebral pathologies [1].

SUSCEPTIBILITY OF DIFFERENT BRAIN REGIONS TO ANOXIA

It has been known that even with several minutes of interrupted oxygen to the brain tissues, there can be significant neurological and neuropsychological changes [6]. The activation of complex cascade and release of expiatory neurotransmitters, in response to anoxia, lead to cerebral edema and brain tissue damage [3,7].

Different areas of the brain possess different sensitivities to anoxia. The sensitivity depends on their metabolic activity, field size of perfusion and neuronal density. Areas of the brain with higher metabolic demands, larger perfusion an high neuronal density are more prone to damage [7]. These vulnerable areas include the CA1 area of the hippocampus, parieto-occipital-temporal cortex, purkinje fibers of the cerebellum and occasionally the amygdala, caudate, lent form and thalamic nuclei [3]. The brain stem, hypothalamus and basal forebrain are less sensitive to anoxia [5]. As perfusion is restored, the brain stem, spontaneous breathing and reflexes return more rapidly while the aforementioned deep brain structures take longer to return to function [8].

CLINICAL MANIFESTATIONS OF ANOXIC BRAIN INJURY

A prospective study from Poland conducted on out-of-hospital cardiac arrest patients, assessed these patients for neurological and neuropsychological sequelae in various time frames. The most frequent early post cardiac arrest impairment was severe consciousness disorders. None of the patients had this amnesia alone; it coexisted with subtle neurological symptoms. Severe motor deficits were observed only in patients with multiple serious cognitive impairments who recovered from severe consciousness disorders. The mechanism and dynamics of functional recovery after cardiac arrest related brain injury remain poorly understood [9].

Literature shows that in mild to moderate cases of cerebral anoxia, the most commonly reported clinical manifestation is memory impairment [5]. In severe cases, patients remain in a permanent vegetative state [10].

Since, the CA1 region of the hippocampus is the most susceptible to damage by anoxia; many

studies attribute this damage with classic amnesic syndrome [2]. A case report where a patient suffered bilateral damage to the CA1 region of hypothalamus, was found to have permanent anterograde and retrograde amnesia [11]. however, in old studies, it is suggested that memory impairment is actually a cause of disturbance in the fronto-subcortical circuits [12-14]. This is supported by a recent study that shows that memory deficits are associated with other cognitive functions and usually follows a pattern of frontal dysfunction [1].

Other neurological and psychological parameters that are usually affected are motor function [1], visual field defects [2], behavior, emotion, language [5], concentration, attention, affective regulation [15], insight, recognition and learning [6].

Many studies suggest that anoxic injury causes frontal dysfunction which causes dysexecutive syndrome, which is often seen in patients with cerebral anoxia [1]. This syndrome manifests as a deficit in executive functions or the ability of the brain to perform complex and multi-step processes such as coordination of bladder and bowel functions. Another common feature in anoxic injury survivors is impaired learning with more difficulty in declarative learning than procedural learning which supports the fact that hippocampus is more affected in anoxia than basal ganglia and cerebellum.

Personality and behavioral disturbances are common following cerebral anoxia [16] which includes lack of empathy, easy distractibility, and lack of inhibition, irritability and child-like behavior. These changes are observed when anoxia affects fronto-basal and fronto-limbic circuits [5].

Other less commonly seen effects of cerebral anoxia are language disturbances and visuo-spatial deficits [1,5]. The most common language deficit observed is expressive. Visuo-spatial deficits are often associated with other cognitive disorders [1]. Visual agnosia and cortical blindness occur as a result of damage to areas of the brain controlling visual scanning, perception and attention [5].

Motor functions are affected depending on the level of damage to the basal ganglia and cerebellum which are not as vulnerable to anoxic damage as compared to areas controlling memory and cognition. Such disturbances affect gait, articulation, posture, dysarthria, dysphagia and movement disorders including rigidity, chorea and ataxia [10,16].

CLINICAL MANIFESTATIONS OF BRAIN DEATH

According to Shutter, almost every function performed by the brain can be divided in 3 main functional categories: Cognitive, hormonal and integrative functions.

In a discussion of brain death, it is crucial to differentiate between awareness and arousal. Arousal is when a human being react to a stimuli in a physical and psychological state. Arousal can be experienced without awareness, but awareness, to react to stimuli, cannot be achieved without

arousal. Patients with anoxic brain injury, who can be aroused without awareness are said to be in a vegetative state. This is loss of cognition and higher cortical function and indicates "higher brain death".

Hormonal balance is controlled through a feedback loop between the brain and endocrine system through the hypothalamus-pituitary axis. The hypothalamus produces releasing factor hormones that subsequently stimulate the pituitary gland to release hormones to stimulate the respective endocrine glands. There are several environmental factors, such as stress and seasonal changes that affect the endocrine system. The feedback system helps to demonstrate healthy brain function to respond to environmental factors and is definitely not mandatory for survival; this is because, in an event of cerebral anoxia, damage to the hypothalamic-pituitary axis can be compensated, through the use of medications that are hormonal replacements. Moreover, endocrine glands can continue to release hormones independent of brain function. A patient sustaining cerebral anoxia, with loss of higher cortical function, brainstem reflexes and regulation of hypothalamic-pituitary axis, is diagnosed to have "whole brain death". This concept, is however, not accepted globally because the feedback loop system of hormonal control is not completely dependent on brain functionality [17].

The loss of integrative function prevents a brain to generate physiological responses that maintain homeostasis and protective reflexes. The brainstem integrates the most basic reflexive protective responses. Anoxia affecting the brainstem can result in the loss of brainstem reflexes that are vital in maintaining cardiopulmonary function. Loss of brainstem integrative functions is considered as death by neurological criteria, or brain death by the Uniform Declaration of Death Act [18]. In fact, to determine brain death by demonstrating brain stem reflexes are a part of the neurological criteria set by the American Academy of Neurology [19].

DETERMINATION OF BRAIN DEATH

The President's Commission study drafted The Uniform Declaration of Death Act in 1981, [20]. which was approved by both the American Medical Association **(AMA)** and the American Bar Association **(ABA)**. The act states "An individual who has sustained either

1) Irreversible cessation of circulatory and respiratory functions, or

2) Irreversible cessation of all functions of the entire brain, including the brain stem, is dead. A determination of death must be made with accepted medical standards [21].

The UDDA has been adopted by most US states as a guideline to determine brain death. Several states have added certain amendments such as the determining physician's qualifications, another physician's confirmation and religious exemptions [22].

In 1995, the American Academy of Neurology published a practice parameter describe the medical standards to diagnose brain death [23]. According to this parameter, to confirm

irreversible clinical brain and brain stem death, 3 clinical findings were emphasized: coma (with a known cause), absence of brain stem reflexes and apnea.

Despite publication of straightforward clinical findings, practice variations remains to this day. Variations were found in prerequisites, number of required physical examinations to draw a conclusion, lowest acceptable core temperature etc [24]. To discuss variations is beyond the scope of this chapter which aims to concentrate on the standard neurological criteria of determining brain death.

According to the American Academy of Neurology guideline, determining brain death can be divided into 4 steps:

Clinical Evaluation (The Prerequisites)

Evaluate irreversible cause of coma

To determine brain death, first and foremost, an irreversible neurological process must have occurred that is compatible with the loss of brain and brain stem function. This can usually be established by history, clinical examination, neuro-imaging and laboratory tests.

Effects of a CNS depressant drug or an electrolyte abnormality affecting brain function should be excluded. This will include a series of laboratory tests such as drug screen, clearance calculations using five times the drugs half-life (assuming normal hepatic and renal functions), drug plasma levels below therapeutic range. Other factors to be considered are prior use of therapeutic hypothermia for cardiac arrest may delay the metabolism of drug and blood alcohol level (levels below the legal limit for drivers are satisfactory to proceed with the clinical evaluation to determine brain death).

Any recent administration or presence of neuromuscular blocking agents should be excluded. This is done by stimulating the ulnar nerve. Presence of a series of 4 twitches indicates absence of any such drug.

Any electrolyte, hormonal or acid-base imbalances should also be excluded, including severe acidosis or significantly abnormal laboratory values.

Normalize core body temperature

Warming blanket may be used to raise the body temperature and maintain the normal core temperature (>36 C).After the initial equilibration of arterial carbon dioxide **(CO2)** with mixed central venous CO2, the partial pressure of CO2 **(PaCO2)** rises steeply, but then more slowly when the body metabolism raises PaCO2. To avoid delaying an increase in PaCO2, normal or near normal core temperature is preferred during the apnea test.

Normalize systolic pressure

Loss of peripheral vascular tone and hypovolemia due to endocrine disturbance (diabetes

insipidus) can cause hypotension which is commonly observed in patients suffering cerebral anoxia. Vasopressors or vasopressin may be given to normalize systolic blood pressures. Neurologic evaluation is considered reliable with a systolic blood pressure of > 100mmHg.

Perform ONE neurologic examination (Sufficient to pronounce brain death in most US states)

If it is confirmed that a certain time period (usually several hours) has passed to recover from brain injury, only one neurologic examination is sufficient to make the clinical diagnosis of brain death. This is practiced in most states, while some US states require two neurological examinations. However, it seems reasonable to inquire if the physician diagnosing brain death possesses the relevant expertise, qualifications and familiarization with the criteria for brain death. This can differ in US by state or institution. Legally, all physicians are allowed to diagnose brain death in most US states.

Neurological Assessment

Coma

There should be lack of all evidences of responsiveness, that is, no eye opening or eye movement on painful stimuli. No motor response (other than spinal reflexes) generated on a painful stimuli. There is a required level of expertise to differentiate between spinally mediated motor response or retained motor response associated with brain activity [25].

The painful stimuli are usually a noxious deep pressure to core body structures. Appropriate locations include the supraorbital notch, mandible at the ankle of the jaw, upper trapezius, anterior axillary fold and the sternum. Pain applied to peripheral locations such as the nail beds may produce spinal mediated reflex confusing the response with brain activity. The noxious deep pressure painful stimuli specifically tests responsiveness of the corticospinal, rubrospinal and vestibulospinalmotor pathways [17].

Absence of brain stem reflexes

Pupillary reflex (CN II): A bright light should be shined into each eye. In a patient with brain death, the pupils are usually dilated (4-9mm), fixed and non-responsive to this stimulus. Constricted pupils will suggest the possibility of drug intoxication.

Oculocephalic reflexes (CN III, IV and VI): Oculocephalic reflexes help us examine the response of CN III (oculomotor), CN IV (trochlear) and CN VI (abducens) to head movement, also known as "Doll's eye reflex" which is elicited by head movement rapidly in a side-to-side motion. Functional CNs will cause the eyes to move in the direction of the head movement to maintain the forward gaze, however, in a person with brain death, there will be no eye movement with head movement [25].

Oculovestibular reflex (CN VIII): This reflex is also referred to as "cold calorics" and is used to

test CN VIII (vestibular).The head of the bed is placed at a 30° angle to allow maximum stimulation of the semicircular canals. The tympanic membrane is visualized to confirm patency of the external auditory canal. The eyes are held open. Each ear is then irrigated individually with 60 mL of ice water and observed for a minute. In a normal integrative response, a slow movement of the eyes towards the irrigated ear is observed. Both eyes need to be tested by introducing ice water in both ears and the examinations should be separated by a few minutes. In a person with brain death, there will be no movement of the eyes.

Corneal reflex (CN V and VII): Corneal reflex is elicited by touching the sclera with a tip of cotton plug to stimulate the CN V (trigeminal). The CN V then activates CN VII (facial) which in turn stimulates the orbicular is oculi muscle and causing the eye lid to shut down. Each eye should be tested individually. Lack of this response indicates brain death [17].

Absence of facial muscle response to a painful stimulus, which would normally produce a grimace, is seen in a person with brain death.

Gag reflex (CN IX): The gag reflex is produced by the CN IX (glossopharyngeal). This is elicited by stimulating the posterior pharynx using a tongue blade, a suction device or moving the endotracheal tube. In brain death, the gag reflex is absent.

Cough reflex (CN X): To assess the responsiveness of CN X (vagus), a suction catheter is pushed through the endotracheal tube to the level of the carina. This movement produces a cough reflex that is otherwise absent in a patient with brain death.

Apnea testing

The final component of brain death examination is the apnea testing. This examination evaluates the person's breathing drive. This is done by performing a CO2 challenge to document a rise in the PaCO2.

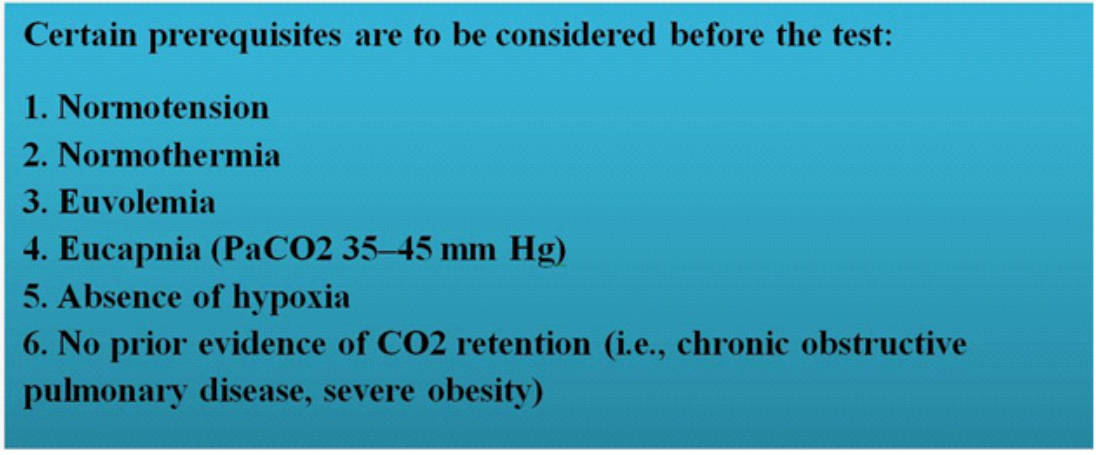

Figure 1: The final component of brain death examination is the apnea testing.

Next step is to adjust the ventilator to achieve a normal CO2 level of 35 to 45 mm Hg (or the patient's baseline, if there is presence of a pulmonary disease with CO2 retention) with a positive end-expiratory pressure of 5 to 8 cm H2O. Preoxygenation with 100% FIO2 is provided for a minimum of 10 minutes to achieve a target PaO2 > 200 mm Hg. Number of breaths of the ventilator is reduced to 10 breaths/min to eucapnia.

A baseline arterial blood gas **(ABG)** test is performed to confirm if the aforementioned goals are achieved. The ventilator is then disconnected from the patient. The oxygenation is however preserved by delivering 100% O2 at 6L/min through an insufflation catheter via the endotracheal tube and close to the level of the carina.

Respiratory movements (effective movement of the abdomen or chest excursions) are then observed for 8 to 10 minutes. The respiratory movements may include a brief gasp.

The apnea test should be aborted if the systolic blood pressure falls < 90mmHg or if the oxygen saturation decreases to <85% for a period of greater than 30 seconds. The procedure may be retried by using a T-piece continuous positive airway pressure 10cm H2O and 100% O2 12L/min.

After the observation period of 8 to 10 minutes, if no respiratory drive is detected, repeat arterial blood gas with base excess. In the absence of respiratory movements and arterial PCO2 of ≥60 mm Hg (or 20 mm Hg increase in arterial PCO2 over a baseline arterial PCO2 for that patient), the apnea test result is concluded as positive and the person diagnosed clinically of brain death).

If the test is unable to conclude brain death and the patient is hemodynamic ally stable during the procedure, the test may be repeated, this time for a longer period of time (10-15 minutes) after waiting until the patient is adequately preoxygenated.

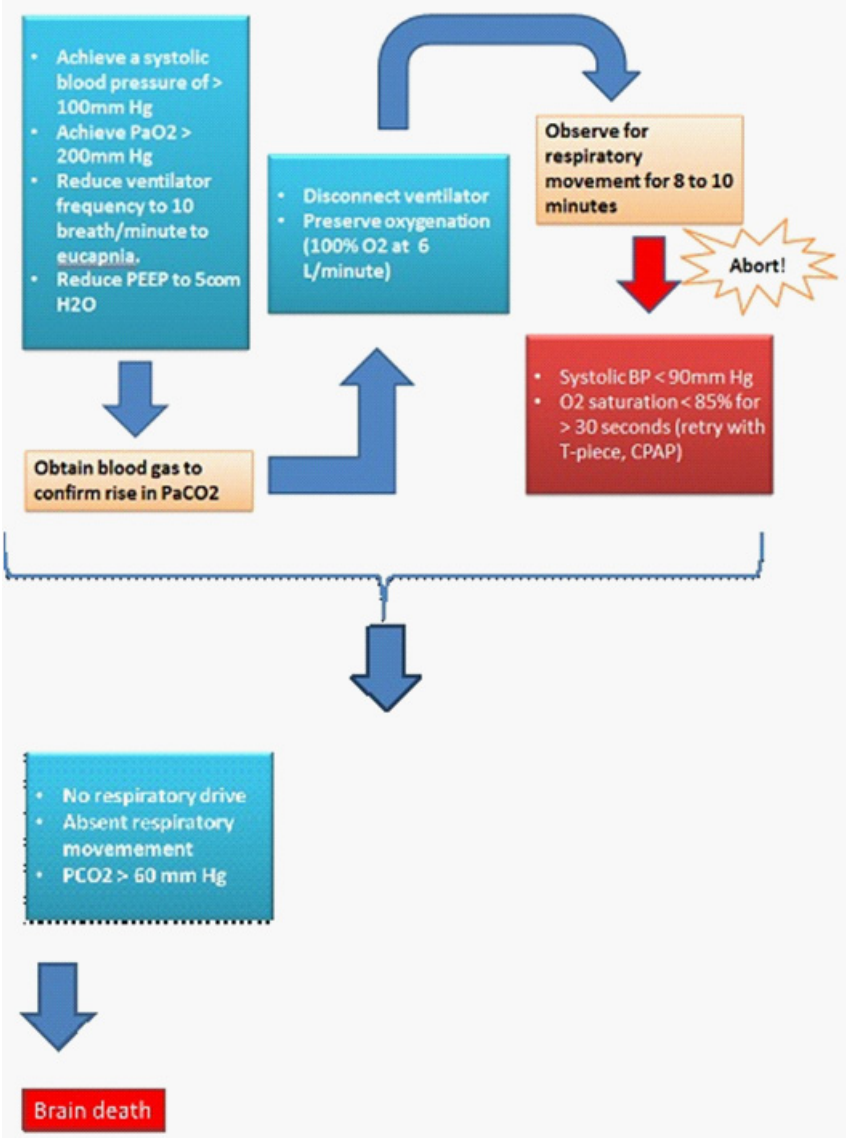

Figure 2: Apnea test.

Ancillary Tests

Some hospitals perform tests such as EEG, cerebral angiography, nuclear scan, and transcranial Doppler **(TCD)** as ancillary tests if they are uncertain about the reliability of neurological examinations or unable to perform apnea test. Some guidelines suggest ancillary tests to shorten the duration of observation period. According to AAN, ancillary tests are not needed for diagnosis of brain death and cannot replace neurologic examination. It is suggested that instead of ordering ancillary tests, physicians should not conclude with the diagnosis of brain death if sufficient clinical evidence is unavailable [25,26].

PATH PHYSIOLOGY

Cardiac arrest causes global brain ischemia, which initiates neuronal death. The primary point of CPR is to restore adequate blood flow to supply the brain and cardiac tissue with oxygen. The mechanism of ischemic brain damage has been investigated with the use of several animal models. Literature suggests the idea that effective CPR could bring about broad ischemia/reperfusion injury to the brain that is firmly associated with poor neurological outcomes [27].

The perfusion shortfall can be characterized precisely by onset of cardiac arrest and cessation of the blood flow to the brain. Unless in circumstances of an observed cardiac arrest, the period of cerebral ischemia and anoxia is unknown. The degree of neuronal damage is influenced by the length of the circulatory arrest. Fifteen minutes of cerebral ischemia due to cardiac arrest can risk ninety five percent irreversible neuronal damage [28].

Cessation of Aerobic Respiration and Glutamate- Ca^{++} Hypothesis

Intracellular acidosis develops within seconds of cardiac arrest due to oxygen insufficiency. Mitochondrial oxidative phosphorylation ceases, exhausting Adenosine triphosphate **(ATP)**, the energy currency of metabolism [29]. Na^+- K^+ ATPase cell membrane exchanger begins to function ineffectually. The levels of extracellular K^+ and intracellular Na^+ spurt up, leading to cellular ionic disequilibrium. Raised intracellular Na^+, increases the intracellular Ca^{2+} levels. Further damage ensues as, influx of $Ca2^+$ ions through voltage gated $Ca2^+$ channels leads to release of glutamate from pre synaptic vesicles through a $Ca2^+$ dependent mechanism [30,31]. Release of glutamate, occurs when the glutamate transporters function oppositely. This happens as a result of ineffectual Na^+-K^+ ATPase functioning across the plasma membrane, in brain ischemia [32,33]. A single glutamate vesicle released, initiates an excitatory post synaptic potential, which is primarily due to the activation of α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid **(AMPA)** receptors. Endogenous glutamate discharged from neurons can add to acute neurodegeneration. Glutamate can be neurotoxic through an agonist impact on N-methyl-D-aspartate **(NMDA)**, AMPA, kainate or Group I metabotropic receptors. Acute neuronal cell demise after transient global or focal brain ischemia is by all accounts dependent on both NMDA and AMPA receptors.

The essential mechanism behind neuronal cell death, is ionic disequilibrium related to the raised intracellular levels of Na^+ and $Ca2^+$ through ligand-gated and voltage-sensitive channels [34,35]. Glutamate receptor activation causes elevation of intracellular calcium due to Ca^{2+} influx through the AMPA, NMDA and voltage gated Ca^{2+} channels. Also, the activation of metabotropic glutamate receptors initiates IP3 production which leads to IP3 receptor activation on the endoplasmic reticulum **(ER)** membrane and subsequently expulsion of Ca^{2+} ions from ER into the cytoplasm. This raised Ca^{2+} ion level in the cytoplasm promotes Ca^{2+} intake by mitochondria. Excessive calcium ion levels in the cytoplasm result in production of reactive oxygen species **(ROS)** and halt ATP synthesis. The ROS which are produced as a result of glutamate induced Ca^{2+} intake include hydrogen peroxide, peoxynitrite, superoxide anion and hydroxyl radicals.

The mechanism behind neuronal death by calcium includes several cascades [36]. Ca^{2+} induce calpains activation which are cysteine proteases that destroy membrane receptors, metabolic proteins and cytoskeleton [37]. They also activate caspases and thus trigger apoptosis. Apoptosis is initiated by the activation of pro apoptotic proteins like p-53, Bax and Par-4 by calcium, resulting in mitochondrial membrane dysfunction. Calcium increases cellular oxidative stress. The mechanisms include oxygenase induction as in arachidonic acid metabolism process and membrane lipid per oxidation [36]. The Glutamate-Ca^{2+} hypothesis of excitotoxic neuronal cell death is generally well acknowledged. As per this hypothesis, the most vital part of the mechanism of cerebral ischemia is the confinement of energy substrates and oxygen to the mitochondrial respiratory framework and the cellular ATP depletion. The cessation of cellular energy production induces acute neuronal cell death [30].

Inflammation

Inflammatory cascade begins within seconds of brain ischemia. Interleukin-1 **(IL-1)** is one of the leading cytokine involved in regulating inflammatory reactions. It is accredited as a prototypic inflammatory cytokine. Though it is not detected easily in plasma but its CNS mediated effects on inflammation have been well-known [38].

The ischemia starts a series of inflammatory events which occur in the brain, its circulation and the lymphoid tissues. The events begin in the vasculature, followed by the formation of reactive oxygen species. Changes in sheer stress, block the arteries by initiating the coagulation pathways ensuing in complement, platelet and endothelial cells activation [39]. Two types of cells play part in releasing inflammatory cytokines. One type are the circulatory cells which include neutrophils, monocytes and macrophages and the other type are the local occupant cells like microglia, astrocytes and endothelial cells. Leukocytes are activated and infiltrated from the periphery. Endothelium is also activated which results in ROS initiation and damage of tissue and endothelium by oxidative stress [40].

Meanwhile, fibrin is formed causing micro vascular occlusion by entrapping the leukocytes and the platelets. Pro inflammatory signals are produced by the adhesion molecule P-Selection, which gets attached to the platelet membrane and endothelium within minutes of ischemic insult. The oxidative stress in the endothelial cells leads to reduction in Nitric Oxide **(NO)** availability. NO is a local vasodilator which inactivates leukocytes and aggregation of platelets. As the NO levels are depleted, this results in further worsening of the vascular occlusion and obstructing the blood flow to the injured zone. Another factor which adds to the micro vascular obstruction is the pericyte constriction due to the oxidative stress. The Blood Brain Barrier **(BBB)** gets damaged by inflammatory mediators and oxidative stress. Transport across endothelial cells is enhanced due to the elevated pinocytic vesicles in the endothelial cell **(EC)** cytoplasm. Down gradation of junctional proteins of the EC and proteases expression in vascular cells by leukocytes lead to leakage of proteins through the BBB.

Ischemia induced mast cells and macrophages are activated in the per vascular spaces. The mast cells release histamines, proteases and Tumor Necrosis Factor **(TNF)** whereas pro inflammatory cytokines are released by macrophages. All these take part in the production of adhesion molecules by the endothelium and in BBB damage, resulting in extravasation of polymorphoneuclear leukocytes **(PMNs)** [39,41].

The local occupant cells like the microglia are induced by the pro inflammatory cytokines, TNF-alpha, IL-1beta and TGF-beta and adhesion molecules and cytokines [40]. Microglia alter their genetic expression and phenotypic characteristics in acute response to the pro inflammatory stimuli. This leads to induction of cytokines like TNF-alpha, IL-1beta and expression of inducible isoform of nitric oxide synthase **(iNOS)**. Oxidative and Nitrative stress is increased as a result of the production of cytokines and chemokines by the microglial induction. Mitochondria are the most effected organelles by this stress and release of mediators by microglial cells [42].

Neighboring cells are also damaged by these trophic factors and cytokines. The compromised BBB also allowes cytokines to cross through it. Thus the CNS gets damaged by the inflammatory mediators produced both within the brain and outside the brain compartment [38].

Consequently, neurological damage and tissue hypoxia occurs as edema ensues due to the insult of neurons, endothelial dysfunction and vasomotor dysregulation by the inflammatory cascade [27].

CPR INTERVAL

CPR restores blood flow by fifteen to twenty five percent of the normal cardiac output values [43]. The purpose of CPR is to circulate the blood again in an attempt to make the heart beat and supply oxygen to the brain to save it from irreversible deterioration and injury that may be caused by prolonged ischemia [44,45]. With return of blood circulation, the production of reactive oxygen species **(ROS)** enhances. Though ROS are produced as the ischemia commences but with reperfusion, the levels reach a spike and they take part in further cerebral damage [46].

Superoxide and its derivatives produced during the recirculation cause the BBB disruption and decreased vascular tone and their production continues for a substantial time after the reperfusion [47]. Initially they are formed in the extracellular compartments and then found in the endothelial and smooth muscle cells of the circulation [48]. These ROS are mainly generated at the complex 1 and 3 of the mitochondria and this is a property of respiratory chain inhibited - mitochondria and reversed electron flow i.e. reduced oxygen supply. These ROS are responsible for cerebral injury and mitochondrial detriment [49]. Other deleterious effects of ROS include peroxidation of lipids, protein SH- group oxidation and carbonyl formation and negative effects on DNA like breakage of its strands [50].

The ROS produced in mitochondria are Superoxide **(O^{2-})**, Hydrogen peroxide **(H_2O_2)** and hydroxyl group **(OH^-)** [49]. They are also generated by lipoxygenase, cycloxygenase, xanthine

oxidase, cytochrome p^{450}, NO synthase of the endothelium and NOX2 of the NADPH oxidase enzymes. It has been shown in experiments that reduced oxygen levels cause the xanthine oxidizes of the endothelium to produce ROS. The levels of ROS produced by xanthine oxidase pathway reach peak level on recirculation thus taking part in reperfusion injury [51-53].

Coagulation and Inflammation

During CPR, with reperfusion, the mediators like prostaglandins, thromboxane, leukotrienes, protein kinases and eicosanoids are released and in association to that, phospholipase and intracellular Ca^{++} are activated. Then, after a few minutes, protein transcription of IL-1 and TNF-alpha starts. After hours of this, mitogen-activated protein kinase, extracellular signal –regulated protein kinase, c-Jun N-terminal kinase **(JuNk)**, nuclear factor-kappa beta and transcription factors are activated. All these exacerbate the inflammatory response [54].

Inflammatory cytokines initiate the coagulation pathways. Tissue factor is inducted and fibrinogen levels also elevate. IL-6 exacerbates the sensitivity of platelets to thrombin and all this leads to coagulation cascade. TNF-alpha causes depression of thrombomodulin and activated protein C thus suppressing the anticoagulant activity and causing microvascular obstruction [55].

Drug of choice for CPR is adrenaline and its alpha1 and alpha2 adrenergic effects cause vasoconstriction. This may help develop arterial pressure required to supply blood to the myocardial and cerebral tissue but it decreases the flow to the cerebral cortex during the CPR due to decreased flow condition of shock [56]. Beta adrenergic effects of epinephrine cause elevation of lactate levels in the arteries and cause severe systemic acidosis [57,58].

POST RESUSCITATION INTERVAL

Mitochondrial Permeability Transition Pore (mPTP) and apoptosis

The two membranes of the mitochondria have different permeability features. Outer membrane is completely permeable while the inner membrane is partially permeable to solute particles of sizes up to 1500 Da under normal conditions. Increased permeabilization of the mitochondrial membrane is a pathway towards cell death [59].

Mitochondrial permeability transition is the process by which the permeability is increased to abnormal levels and this includes opening of a pore which is named after the process as mitochondrial permeability transition pore **(mPTP)**. It is composed of protein complexes from the outer membrane, inner membrane and the matrix. Adenine nucleotide translocase **(ANT)** from the inner membrane, Cyclophilin D from the matrix and Voltage dependent anion channel **(VDAC)** and benzodiazepine receptor of the outer membrane get attached to each other forming the mPTP. This mPTP leads to ionic disturbance across the membrane and cell swell up bursting the outer membrane because of the limitation of the outer membrane expansion characteristic [60].

During the ischemia, there is Ca^{++} ion overload and decreased ATP levels due to the cessation of oxidative phosphorylation. AMP and phosphate levels peak up and this AMP is catabolized to adenosine and inosine thus all the adenine nucleotides are used up. All these factors like increased phosphate and Ca^{++} levels and depression of nucleotides may cause mPTP opening but the low $_pH$ due to lactate accumulation becomes obstruction in mPTP activation. With reperfusion, the conditions become favorable for mPTP opening. The lactate lowers down as they are dropped out of the cell through lactate/H^+ transporter and the reoxygenation elevates the oxidative stress as ROS are generated and mPTP is opened in this new environment which is a consequence of the reperfusion [61].

In one study, it was proved that mitochondria of different brain areas have different vulnerability to the opening of mPTP by high levels of Ca^{++}. Hippocampus is affected more easily while cerebellar mitochondria are resistant as compared to the former. That is why hippocampus is first to be injured by the is chemia [62] and this difference of response may be due to the mitochondrial overall adenine nucleotide levels [63].

The membrane potential of the mitochondria falls down which may initiate increased production of ROS, diminished ATP and tearing of the membrane due to swelling. Pro apoptotic proteins like Bcl2 migrate to the outer membrane which leads to the activation of cytochrome C, endonuclease G and Smac from the space between the inner and outer membrane. Caspases are activated by the catabolic action of Smac on caspase inhibiting proteins. Caspase 3 and 9 proteases are initiated by the bondage of cytochrome C and apaf 1. Endonuclease G enters the nucleus and breaks down the DNA. All these actions of the proteins cause cell death by apoptosis [64].

The cell death can occur through necrosis in case when the initial ischemic insult is very stringent. The mPTP opening is exacerbated and ATP is depleted to a harsh extent which result in poor ATP generating capacity and the cell demise occurs by necrosis [65]. That is because of the reason that more and more ATP is hydrolyzed with more and more opening of the mPTP and the energy generation is worsened leading to increased Ca^{++} disequilibrium. So this cycle goes on and phosphorylation capacity of the cell is further regressed and Ca^{++} ion control lost, leading to cell demise [66].

CONCLUSION

Literature suggests several mechanisms of post cardiac arrest brain injury but no single mechanism has been authenticated so far. The inflammation cascade and the Glutamate Ca^{++} hypothesis are the main death process initiators. Reperfusion injury after CPR through mPTP has significant importance as this leads to the mitochondrial insult which is the power house of the cell.

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