

ABSTRACT

CNS traumas, and tumors are major health problems. They lead to serious health and economic burdens. They are major causes of disability, or death all over the world. Nowadays, the order of causes of trauma-related death has been changed from multiorgan affection to CNS trauma. Assessment of severity and predicting the outcome in a patient with a head, or spinal cord injury, is a must. Most of severity assessment scales depend on the clinical data of the patient. Using of biochemical, and genetic markers that have correlations with severity and outcome in CNS trauma is necessary of and helpful in clinical field. Ubiquitin C-terminal hydrolase-L1 is an enzyme that is present in the cytoplasm of neurons, and forms about to 1–2% from the proteins found within the human brain, and it is supposed to be a promising biomarker in neurological diseases especially trauma. Neuroglobin (Ngb), is a protein which is distributed mainly in central and peripheral nervous systems, and it has neuroprotective effects against oxidative stress. It is an important biomarker in neurological trauma and tumors. CNS tumors have psychological burden beside their health and economic effects as they may affect personality and both physical and cognitive independence. Various types of mutations have a role in pathogenesis of CNS tumors. Isocitrate dehydrogenase enzyme isoform 1 (IDH1) mutations are frequently identified in primary CNS tumors. Biochemical and genetic markers of clinical importance in CNS trauma and tumors are discussed in this review.

1. INTRODUCTION

Traumatic brain injury (TBI) is responsible for about one-third of all trauma-related mortalities in the USA annually, and it is a major public health problem in Egypt[1]. Traumatic injuries are considered the leading cause of death in people under the age of 46 years[2]. One of the main problems with a head, or spinal cord injury, is assessing the severity. Neuronal damage markers, released in the blood or cerebrospinal fluid (CSF), may provide valuable information about diagnosis and prognosis of these conditions. Protein degradation in secondary brain injury was said to be mediated by ubiquitin, in which ubiquitin C-terminal hydrolase L1 (UCHL-1)

concentrated in neuronal cells, and it is supposed to be a promising biomarker in neuropathology [3]. Neuroglobin (NGB) is a protein monomer that is structurally related to myoglobin, and it has many important functions in protecting the neurons from oxidative hazards[4]. Neuroglobin (Ngb) is responsible for enhancing oxygen transportation through blood-brain barriers, as well as increasing the availability of oxygen in mitochondria. Neuroglobin is responsible for detecting cellular oxygen concentrations like a sensor, and is involved in the detoxification of harmful reactive oxygen, so it supports neuron survival in hypoxic and ischemic conditions. as well as enhancing cell viability within active

cells[5]. CNS tumors can lead to severe disability,

in the form of seizures, poor memory or speech problems, as well as physical dysfunction[6].

Accumulation of multiple genetic and epigenetic alterations in genes and signaling pathways, such as isocitrate dehydrogenase enzyme isoform 1 (IDH1) mutations, is an important cause of CNS neoplastic transformation[7].

2.CNS traumas

2.1.Traumatic brain injuries(TBIs)

A clear, concise definition of traumatic brain injury (TBI) is essential for reporting, comparison, and interpretation of studies on TBI. Traumatic brain injury was defined by, The Demographics and Clinical Assessment Working Group of the International and Interagency Initiative toward Common Data Elements for Research on Traumatic Brain Injury and Psychological Health, as an alteration in brain function, or other evidence of brain pathology, caused by an external force[8]. Many causes can lead to occurrence of traumatic brain injury such as: motor vehicle accidents, falls, blunt trauma[9]. Among all these causes, a motor vehicle accident is considered the leading cause of brain trauma or brain injuries [10]. The mean value of the number of hospitalized patients with fatal TBI in Europe was 235 per 100,000 while in the US this value was doubled [11]. About 50,000 deaths related to TBI occur in the US per year, and 1.4 million TBIs occur globally with a cost of about 60 billion dollars annually. In the Kingdom of Saudi Arabia, an 8-year retrospective

study reported 1,219 patients below the age of 18 years to be diagnosed with TBI [10]. There are many methods to classify head injuries: according to mechanism of trauma (closed or penetrating injury), morphology (fractures, focal intracranial injury, diffuse intracranial injury), or severity of injury (mild, moderate, or severe). Primary brain injury means the immediate brain damage due to trauma e.g., cerebral contusions, shearing lesions, lacerations, and acute subdural or epidural hematomas. Secondary brain injury refers to progressive cerebral oedema, ischemia, and the expansion of cerebral contusions and the surrounding focal oedema, which lead to increase in intracranial pressure (ICP) within the confined skull and can eventually cause cerebral herniation and death [12]. In the Glasgow Coma Scale (GCS) system, the patients with head injury would be classified into three groups: mild, moderate, or severe. Patients with mild injury have GCS scores from 13 to 15. Those with moderate injury have GCS scores from 9 to 12 and the patients with severe injury have GCS scores which are less than 8[9]. Head Injury Severity Scale (HISS) is a five-interval severity scale (minimal through critical) based primarily on initial GCS score. The HISS scale also includes the aspects of retrograde amnesia, loss of consciousness (LOC), and focal neurological deficits for each severity intervals[13]. Then in 2000, HISS classification was modified, by the Scandinavian Neurotrauma Committee (SNC) by classifying head injuries into minimal, mild, moderate, and severe. Minimal head injury is presented by a patient with GCS 15 at admission and with no LOC or focal neurological

deficits. Mild head injury is defined as initial GCS of 14–15, brief LOC (<5 min) and no focal neurological deficits. Moderate head injury defines a patient with initial GCS of 9–13 and/or focal neurological deficits or LOC \geq 5 min after head trauma. Severe head injury includes all patients with an initial GCS score of 8 or below, hence, unconscious patients[14]. The Revised Trauma Score (RTS) is a numeric grading system for estimating the severity of injury. Its parameters include the GCS, systolic blood pressure, and respiratory rate, each giving rise to a score between 0 and 4. The degree of injury severity can be estimated by the total sum of these three parameters, with the highest score is 12, which represents the least severe injury[12]. The Glasgow Outcome Scale-Extended (GOSE) has become one of the most widely used scales for assessment of the outcome after traumatic brain injury as regard global disability and recovery[15]. The Disability Rating Scale (DRS) is a short, efficient, and rapid method for assessing levels of functional disability for traumatic brain injury (TBI) patients [16]. The DRS is an 8-item measure (each rated on a 3–5-point scale) that is summed to give a total score[17].

2.2. Traumatic spinal cord injury (TSCI)

Traumatic spinal cord injury (TSCI) is a high disabling injury; it can lead to many complications such as: damage or loss of sensation and motor function, dysfunction of bladder or bowel and also may lead to multiple organ dysfunction[18]. There are many causes of SCI, including falls (falls from height, simple falls), motor vehicle accidents (MVAs)/motor vehicle crashes, sports-related accidents, violence and other

remaining causes of injury. Motor vehicle accidents (MVAs) and falls are the most common causes of injury accounting for nearly equal percentages[19]. As a result of the expansion of human activities, the incidence of SCI also increased gradually over last years. The incidence varied from 13.019 per million to 163.420 per million people. The cervical level of spine is the most common affected site. Incomplete cord injury is more common than complete injury quadriplegia is more common than paraplegia. Estimations of SCI mortality among developed countries varied from 3.1% to 22.2%, while mortality in non-developed countries ranged from 1.4% to 20.0%[18]. The extent of injury is defined by the American Spinal Injury Association (ASIA) Impairment Scale using the following categories:

- A = Complete: No sensory or motor function is preserved in sacral segments S4-S5
- B = Incomplete: Sensory, but not motor, function is preserved below the neurologic level and extends through sacral segments S4-S5
- C = Incomplete: Motor function is preserved below the neurologic level, and most key muscles below the neurologic level have a muscle grade of less than 3
- D = Incomplete: Motor function is preserved below the neurologic level, and most key muscles below the neurologic level have a muscle grade that is greater than or equal to 3
- E = Normal: Sensory and motor functions are normal[20].

2.3. CNS tumors

CNS tumors are relatively rare, but they represent a significant cause of cancer-related morbidity and mortality especially among children and young adults where they respectively account for approximately 30% and 20% of cancer deaths[21]. Primary CNS cancers can occur at any age and in any site within the central nervous system, but mostly (more than 90%) affect the brain. These types of cancer are major causes of disability and death in all populations[22]. The global incidence of neurological tumors in 2016, was 330 000 patients while 227000 persons died due to the disease. East Asia was the region with the most incident cases of CNS cancer for both sexes in 2016, followed by western Europe, and south Asia. The top three countries with the highest number of incident cases were China, the United States of America (USA), and India[23]. There is not any biological, environmental, or social causes that identified to lead to CNS tumors except ionizing irradiation whether as a therapy, or during wars.[21]. Glioma, with its different types, is considered the most abundant histopathological variety of primary neurological tumors. Others are medulloblastoma, meningioma, glial tumors (e.g., ependymoma, and schwannoma), and finally lymphoma of the CNS[24]. Glioblastoma, which represents the most frequent glial tumor in the brain, often cause death during 24 months after being diagnosed even if all treatment lines were taken[25]. The most common histological types in children involve medulloblastoma, astrocytoma, tumors of germ-cell origin, gliomas of the brainstem, and ependymomas. Good prognosis and increased life expectancy is common in children affected by these types of tumors in

presence of comprehensive treatment strategies[26]. Dividing neurological tumors into different grades relies on four features in the morphology: presence of atypical cells, mitotically active cells, necrotic changes, and presence of microvascular proliferation. In the light of these features mentioned, CNS tumors are graded into:

- Grade I: in which tumor tissues have none of the mentioned features. This category is characterized by increasing in size slowly, being benign, and increased life expectancy.
- Grade II: possess one feature only, such as, atypical cells. Neoplasms of this group increase in size slowly, meanwhile, they have a high recurrence rate, also, could be benign or non-benign.
- Grade III: possess 2 features, such as, atypical cells which are mitotically active. Neoplasms of this group are malignant and mostly have a high recurrence rate as neoplasms with higher grades.
- Grade IV: possess 3 or 4 of the features mentioned before. Neoplasms of this group are not benign at all having a high aggressiveness[27].

3. Ubiquitin c-terminal hydrolase L1 (UCHL1)

3.1. The ubiquitin system: mechanism of action, and function

Ubiquitin is a protein formed of 76-amino acid. It can be added, either singly or as polyubiquitin chains, to residues in a certain protein as a post-

translational modification. The most common residue to undergo ubiquitination is lysine (Lys), but other residues that can be modified include serine, threonine and cysteine side chains, as well as the N-terminal amino group[28]. Ubiquitin targets proteins via a covalent bond between the ϵ -amino group of the Lys residue of the protein and the last carboxylate group of the terminal glycine (Gly76) of Ub through an isopeptide bond. This covalent addition is catalyzed by three enzymes that act in a sequential order. They are known as the E1 Ub activating enzyme, E2 Ub conjugating enzyme, and E3 Ub ligases in an ATP-dependent process. Ubiquitin can be removed from proteins or from polyubiquitin chains through hydrolysis by one of deubiquitinases (DUBs). There are about 100 DUBs in humans, most of them are cysteine proteases, except the JAMM proteases, which need Zn dependent catalysis. The cysteine protease DUBs are subdivided into six groups: the Ubiquitin Specific Proteases (USP), Ubiquitin C-terminal Hydrolase (UCH), Machado-Josephin Domain, Ovarian Tumor Proteases, and the more recently discovered Mindy and ZUFSP groups[29]. The class of UCH has many members in addition to UCH-L1, including UCH-L3, UCH-L5, and BRCA-associated protein-1; however, only UCH-L1 is abundant in the brain[30]. Ubiquitin-proteasome system through its role in protein degradation, is essential for many different cellular processes, such as response to stress, cell differentiation, and transmission of cellular information. Ubiquitin C-terminal hydrolase 1 (UCHL1) is special in that it engages in a variety of functions, being both ligase and hydrolase. Via acting as a hydrolase, UCHL1 can participate in

recycling and removing ubiquitin from damaged proteins., and as a ligase, It binds ubiquitin particles to each other, then it can be utilized to tag certain proteins. to be hydrolyzed later on. UCHL1 key functions include regulating cellular ubiquitin and glutathione levels, and regulating cell cycle[31]. During both physiological and neuropathological situations, UCH-L1 regulates brain protein metabolic processes by directing the proteasome system, taking part in eliminating proteins present in concentrations higher than needed, abnormally folded proteins, or proteins affected by oxidation. [30]. UCHL1 is required for appropriate motor function and is thought to play a role in memory function. UCHL1 is also found in the peripheral nervous system, such as the dorsal root ganglion and trigeminal ganglion neurons. It interacts with proteins in the neuronal cytoskeleton and is essential for axonal integrity and transport. Furthermore, because it is a component of the axonal skeleton, UCH-L1 plays a function in axonal transport. UCHL1 is highly expressed in testes, carcinomas arising from, lung, prostate, mesenchymal tissues, brain, colon, kidney, pancreas, and breast a, in addition to the nervous system. [31].

3.2. Ubiquitin C-terminal Hydrolase L1 (UCH-L1) structure

In the early 1980s, Jackson et al. were the first to report UCH-L1 as a human brain-specific protein, of approximately 27 kDa molecular weight, using high-resolution 2D polyacrylamide gel electrophoresis[32]. UCH-L1 is a globular protein containing a peptidase C12 superfamily catalytic domain with very short N- and C terminal extensions.

There are five crossings of the peptidase C12 polypeptide backbone forming 'Gordian' knot. In the final 3D structure, there are two 'lobes' of α -helices surrounding a tightly-packed hydrophobic core of β -strands. This knotted backbone protects UCH class DUBs from undesired proteasomal unfolding and degradation. When UCH-L1 is bound to ubiquitin, a conformational change takes place to bring the aspartate, histidine and cysteine residues which form the catalytic triad of the active site for hydrolysis into closer proximity and promotes enzymatic activity [28]. Products of abnormal oxidation of lipid, mutagenesis of genes, and modifications of protein structure after being translated, can all affect the structure and function of UCH-L1. [33]. Over expression and/or mutation of UCH-L1 gene has been linked to various cancers and neurodegenerative diseases [34].

3.3. Biological roles of UCHL-1 in the CNS

3.31 Antioxidant function of UCHL-1 in neurons

It has been proposed that UCH-L1 has a neuronal antioxidant function because the presence of insoluble or misfolded UCH-L1 has been approved in many neurodegenerative disorders [34]. One hypothesis supporting this idea is that the conserved Cys152 residue acts as a redox buffer and reacts with, and chelates, free radicals in neurons [35].

3.32 Ubiquitin C-terminal Hydrolase L1 (UCH-L1) in CNS trauma and neuronal injury

Ubiquitin-proteasome system plays a major role in repairing injured axons and neurons through its function in removing abnormal proteins. After brief ischemia, this is critical because abnormal ubiquitin proteins accumulate within neuronal tissues, leading to cell death. UCHL1 is important for maintaining axonal integrity and axonal transport. Synapses are also regulated by UCHL1. Axon pathology and significant abnormalities in motor functions are caused by UCHL1 mutations. [31]. Ubiquitin UCHL1 has been approved as a serum biomarker for severe traumatic brain injury (TBI). Ubiquitin C-terminal hydrolase-L1 (UCHL1) levels were measured in sera and CSF samples from patients with heat trauma affecting the brain, and put in comparison with non-traumatized controls in many clinical trials. These investigations found a considerable elevation in UCH-L1 levels in the acute phase (within 24 hours) and a link between the concentration reached and the severity of the damage of brain tissue. Also, studies on rat models of TBI revealed that the protein's expression has increased [30].

3.33 Ubiquitin C-terminal Hydrolase L1 (UCH-L1), and Oncogenesis

Abnormal levels of UCHL1 are related to cancer development. UCHL1 activity in tumorigenesis has been linked to cell cycle regulation, possibly, by targeting p53, β -catenin and Akt pathways. UCHL1 activates Akt-mediated signaling depending on its hydrolase activity and subsequently promotes migration, invasion and proliferation of cells. UCHL1 can be produced in malignant tumors originating from tissues that do not express it under normal conditions,

such as breast, colorectal and pancreatic cancers. Contrary to the previous studies other researchers demonstrated that UCHL1 triggered the arrest of cell cycle and inhibited proliferation of tumor cells in hepatocellular, breast and ovarian cancers[31]. In neuroblastoma, high levels of UCHL1 correlated with its differentiation and according to Gu et al UCHL1 could be a marker of good clinical outcome[36].

3.34 Ubiquitin C-terminal Hydrolase L1 (UCH- L1), and neurodegeneration

Presence of ubiquitinated protein aggregates is a common hallmark in many neurodegenerative disorders, like parkinsonism, and Alzheimer's disease (AD). These pathologies have been related greatly to abnormalities of UCHL1 gene. UCHL1 is present in bodies of Lewy and neurofibrillary tangles. The activity of UCHL1 is suppressed by ROS generation, which has an impact on the function and survival of neurons. UCHL1 levels were shown to be lower in Alzheimer's disease and amyotrophic lateral sclerosis patients.[37].

3.4. Other roles of UCHL1 in different tissues

3.4 1 Ubiquitin C-terminal Hydrolase L1 (UCH- L1), and spermatogenesis

Ubiquitin-proteasome system plays a role in all different stages of spermatogenesis, so disturbances of its balance can lead to many abnormalities. UCHL1 has a diverse action during spermatogenesis; It participates in the activation of germ cell death and also regulates anti-apoptotic factors during spermatogenesis. Increased levels of

UCHL1 are linked to a higher rate of germ cell death, most likely due to removal of ubiquitin residues from p53 leading to increased activity of apoptosis through the mitochondrial pathway[38].

3.4 2 Ubiquitin C-terminal Hydrolase L1 (UCH- L1), and angiogenesis

UCHL1 mediates vascular remodeling and has a role in angiogenesis in endothelial cells through increasing the expression of eNOS (endothelial nitric oxide synthase), and inhibiting NF-k (Nuclear factor k) activity mediated by TNF (Tumor Necrosis Factor), which may improve ischemic vascular diseases. UCH-L1 also promotes tubule development in vitro and blood vasculature formation in vivo [31].

3.4 3 Ubiquitin C-terminal Hydrolase L1 (UCH- L1), and inflammation

Following stimulation with proinflammatory cytokines IFN- and TNF-', UCHL1 was found to be increased in multipotent mesenchymal stromal cells (MSCs). In patients with acute appendicitis, the amount of UCHL1 in the plasma was highest before the surgical procedure and gradually reduced afterward. The metabolic response to inflammation may be reflected in UCHL1 levels. [39].

3.4 4 Ubiquitin C-terminal Hydrolase L1 (UCH- L1), and skeletal muscle cell differentiation and proliferation

Animal studies revealed that UCHL1 is primarily expressed in type I muscle fibers, and it has a role in slow twitch muscle growth and function which are

regulated through its action. In vivo and in vitro, deletion of UCHL1 resulted in muscular hypertrophy. [40].

3.5. Human UCHL-1 mutation

A missense (Glu7Ala) point mutation in UCH-L1 has been identified as the cause of early onset neurodegeneration in three siblings who appeared normal at birth, but became blind at 5 years old and also suffered progressive cerebellar ataxia, upper motor neuron disorder, involuntary abnormal eye movements, dorsal column dysfunction, rigidity. No other manifestations were seen outside of the nervous system[41].The Glu7 residue in UCH-L1 is needed for ubiquitin binding. The ataxic phenotype noticed in humans expressing ubiquitin binding/hydrolyzing deficient UCH-L1 suggests that the axonal degeneration observed in the mouse models are probably due to loss of this function[31].Mice lacking the UCHL1 gene had gradual motor disability as well as anomalies in the synaptic connections of corticospinal neurons[42].

4. Neuroglobin (Ngb)

4.1. Overview

Globins are heme-containing proteins that can be found in all living beings of the animal kingdom. They play a crucial function in the creation of oxidative energy. [43].Over many decades, haemoglobin, and myoglobin had been known as the only globins present in vertebrates. But in 2000, a third globin type was discovered by mining the genome sequence data. Based on a preferential expression in the nervous system, this globin was referred to as

neuroglobin[44].Neuroglobin (NGB) is a protein which is structurally related to myoglobin and plays a crucial redox-dependent defensive function in neurons[45]. Ngb is a monomer formed of 150 amino acid residues and weights 17 kDa and its expression is induced mainly by hypoxia such as in case of decreased cerebral or spinal blood supply. It has different concentrations according to the tissue. For example, Ngb concentrations in rods and cones of the retina can be up to 100 times higher than in neuronal cells of the nervous system because they have a high need for oxygen.[46].

Cytoglobin, globin X, which is seen in some murine and amphibian creatures ,and globin E, which is present in bird eyeballs allare different types of the globin family [47]. The last globin found, cytoglobin (Cgb), has 190 amino acids arranged in two chains in a dimeric configuration. It can be present in the heart, lungs, liver, and stomach, and among other organs [46].

4.2. Neuroglobin structure

Globins are globular metalloproteins that have a common tertiary structure characterized by six or four α -helices (3/3 or 2/2-fold symmetry, respectively) that make a sandwich around a heme group. They bind O₂ in a reversible manner via an iron-containing porphyrin ring. Neuroglobin (Ngb) has the same common globin structure consisting of eight α -helices denoted A–H, with the

heme prosthetic group located between helices E and F. But it differs in structure from hemoglobin and myoglobin as the heme iron (heme-Fe) of Hb and Mb proteins is pentacoordinated, but the heme-Fe of Ngb proteins is hexacoordinated by four pyrrole nitrogen atoms within the heme plane, the distal His(E7)64 side chain, and the proximal His(F8)96 amino acid particles[43].

4.3. Neuroglobin functions

Neuroglobin (Ngb) is responsible for increasing oxygen transportation through blood-brain barrier (BBB), as well as increasing the availability of oxygen in mitochondria. Neuroglobin acts as a sensor that detects cellular oxygen concentrations, and is involved in the detoxification of harmful reactive oxygen, so it supports neuron survival in hypoxic and ischemic conditions, as well as enhancing cell viability within active cells. Neuroglobin (Ngb) may be involved in a signal transduction pathway, e.g. by inhibiting the dissociation of GDP from G protein α . Ngb may be part of a redox process that is for example instrumental in preventing apoptosis via reduction of cytochrome [44]. It's also been postulated that Ngb's neuroprotective characteristics are linked to its ability to interact with mitochondrial cytochrome C (Cyc), rather than only its free radical scavenging ability.[48].

4.4. Reaction of Ngb with reactive oxygen and nitrogen species

Not only Hb(II)-O₂ and Mb(II)-O₂ have the ability of rapid reaction with nitric oxide (NO) to produce nitrate and Hb(III) or Mb(III) but also human Ngb(II)-O₂ can play this scavenging role. Human Ngb(II)-O₂ reaction with NO is very rapid and proceeds *via* a peroxynitrite intermediate which bounds transiently to Ngb(III) [49]. Neuroglobin was proposed to play a similar function to that of Mb, acting as a NO-dioxygenase when PO₂ is decreased (e.g., after an ischemic insult) and NO levels are elevated. Therefore, depending on the oxygen partial pressures, Ngb may either decompose or produce NO, which may be instrumental in the control of vascular tone as regard contraction and relaxation, and in controlling the level of mitochondrial respiration [50]. As a result of the increased O₂ consumption process, mitochondria may produce various harmful ROS. Such process is most observed under hypoxia, when the flow of O₂ is decreased. Globins are suggested to have the ability to protect the cells from ROS stress and Ngb may have a similar role in neuronal cells [44].

4.5. Neuroprotective properties of neuroglobin

Neuronal tissue is very sensitive to hypoxia-related stress. Any protein that can enhance O₂ supply or that depress the reactive oxygen species (ROS)-induced injuries is expected to enhance cell viability under ischemic stress. In agreement with this, many studies had demonstrated that experimentally

decreased Ngb levels reduced, whereas overexpression of Ngb improved survival of cultured neurons [51,52].

Ngb is linked to microdomains of lipid rafts and α -subunits of heterotrimeric protein G and can be activated during oxidative stress, with structural changes that lead to neuroprotection. Moreover, Ngb, which is present in the mitochondria, reacts with the voltage-dependent anion channel (VDAC) and cytochrome c, which illustrates the function it does in protecting nerve cells on the mitochondrial level. [53]. It has been shown that Ngb reduced amyloid- β (A β) protein toxicity in mouse neurons and pheochromocytoma cell lines suggesting that its antioxidant properties could protect against AD. Many experiments, carried out on AD patients' brains after death, revealed that Ngb expression in hippocampus tissue was upregulated during the early and moderate phases of the disease, but Ngb concentrations fell to control levels in late AD[43]. In another research, it was hypothesized that The activation of Ngb via the PI3K/Akt pathway reduces caspase action, allowing cells to survive, which could be an alternate technique for preventing AD progression [54]. Other studies have shown that neuroglobin reacts very rapidly with cytochrome c released from mitochondria during cell death, thus interfering with the intrinsic pathway of apoptosis [55].The potential neuroprotective function of Ngb has been also investigated in Huntington's disease (HD) [43]

4.6. Neuroglobin and CNS trauma

Basic pathologic criteria of TBI include bleeding, cell death, increased production of β -amyloid, basic fibroblast growth factor (FGF-2), and overexpression of Ngb that remains upregulated until the sixth day post-injury. Neuroglobin expression is increased also with stroke, hypoxia, and ischemia [56].In an experimental model of trauma induced injury of the brain, increased Ngb expression was associated with a noticeable decrease in sensorimotor impairments when compared to controls that did not overexpress neuroglobin[57].On the other hand, measurement of Ng levels in patients with traumatic brain injury revealed a time-dependent increase of neuroglobin levels in serum of patients with TBI within the 96 h post-injury also it was observed that upregulation of neuroglobin correlated to severity and prognosis of TBI as serum NG levels significantly increased in TBI patients with bad outcomes [58].

As regard spinal cord trauma many studies showed that over expression of neuroglobin is associated with good recovery from the spinal cord injury. Overexpressing Ngb improved the recovery of locomotor function after spinal cord injury significantly. Such effect may be due to the inhibition of neural apoptosis through the mitochondrial pathway[59].

4.7. Neuroglobin and CNS tumors

Cancer is associated with increased oxidative stress due to the continuous proliferation and metabolism of cancer cells. But on the other hand, cancer cells have an intrinsic higher antioxidant capacity when compared to the normal cells so, they are able to cope with oxidative stress-induced cell death. The identification of survival factors and adaptive pathways in cancer cells against oxidative stress, provides many targets for the promising therapeutic intervention against cancer. Neuroglobin (NGB), being an oxidative stress sensor and cytoprotective factor against redox imbalance, has been considered as a new tumor-associated protein [60]. Different researchers studied the expression of human NGB in the nervous system neoplasm. They found that NGB expressions was higher in the mouse and the human astrocytoma cell line and in human astrocytoma tissues when compared with normal astrocytes, indicating a possible role of NGB in the adaptation of astrocytoma to the hypoxic and oxidative stress conditions [61]. Also, the analysis of both NGB mRNA and protein levels showed an up regulation of NGB levels in glioma tissue with respect to the normal tissues. There was a correlation between NGB expression and the worse clinic-pathological feature, type/grade of glioma, poor prognosis, and shorter survival overall which led to proposing NGB as a prognostic marker for glioma patients [60].

5. Isocitrate dehydrogenase I (IDH1)

5.1. Overview

It is known that, the tricarboxylic acid (TCA) cycle (named by the citric acid cycle or Krebs cycle also) is used by all aerobic organisms to produce adenosine triphosphate (ATP) from acetyl-CoA obtained from carbs, lipids, and proteins through. The TCA cycle also produces intermediate products that can be employed in denovo protein, lipid, and nucleic acid synthesis. [62]. Isocitrate dehydrogenases (IDHs) catalyze the oxidation-decarboxylation of isocitrate (ICT) into -ketoglutarate (-KG) utilizing NAD or NADP as a cofactor, which is one of a series of metabolic events in the TCA cycle. NADP-dependent cytoplasmic IDHs are found in most prokaryotes (NADP-IDHs). NADP-IDHs and NAD-dependent IDHs both exist in Eukaryotes (NAD-IDHs). Two types of NADP-IDHs are found in the cytoplasm and mitochondria of human and other mammalian cells, and one NAD-IDH is found in the mitochondria. HsIDH1, HsIDH2, and HsIDH3 are the human NADP-IDHs and NAD-IDHs, respectively. [63]. While all the three types of IDH catalyze similar enzymatic reactions, they have different cellular functions and localization. IDH1 and IDH2 exist as homodimers which reduce NADP⁺, while IDH3 is a structurally distinct enzyme which utilizes NAD⁺ as a cofactor. IDH2 and IDH3 are mitochondrial enzymes which play critical roles in generating

mitochondrial NADPH and driving TCA cycle flux, respectively. In contrast, IDH1 is localized primarily in the cytosol where it plays an important role in maintaining cytosolic NADPH levels [64]. HsIDH3 is recognized to play a catalytic role in the citric acid cycle, whilst HsIDH1 and HsIDH2 play a pivotal role in cellular protection from oxidative stress hazards, oxygen free radicals' elimination, lipogenesis and cholesterol synthesis. The development of a variety of metabolic disorders and malignant tumors has been linked to abnormal activities of all these three enzymes.[63].

5.2. Structure, and regulation of IDHs

5.2.1 NADP-IDHs

NADP-IDHs are found in both prokaryotes and eukaryotes and function as homodimers with catalytic activity in both subunits. The catalytic mechanisms of these enzymes are similar, but differ in regulation of catalytic processes[65]. Prokaryotic and eukaryotic Kinases and phosphatases regulate critical signal transduction in cells by reversibly phosphorylating and dephosphorylating proteins. They predominantly act on hydroxy amino acids tyrosine, threonine, serine particles of proteins [66].

Isocitrate dehydrogenase kinase/phosphatase (AceK) is a peculiar bi-functional enzyme in *Escherichia coli* that functions as both a protein kinase and phosphatase. AceK responds to

dietary diversity by addition or removal of phosphate to / from isocitrate dehydrogenase (IDH)[67].The reversible phosphorylation results IDH, which governs the junction in-between the Krebs and the glyoxylate bypass cycles, can lose its activity or become activated by addition of phosphate reversibly. Furthermore, as compared to its kinase and phosphatase actions, AceK has an exceptionally robust ATPase function[68].The double functioning kinase/phosphatase AceK controls NADP-IDH activity by reversibly phosphorylating and dephosphorylating a strictly defined Ser in the catalytic site, and other bacterial NADP-IDHs may have the same regulatory mechanism.[63].The performance of human IDH1 is controlled by a morphological shift in a critical structural component in the catalytic site triggered by substrate binding, and perhaps other mammalian NADP-IDHs may use an analogous regulatory mechanism[69].

5.2.2 NAD-IDHs

- **Prokaryotic NAD-IDHs**

Saccharomyces cerevisiae is a kind of yeast which harbor Prokaryotic NAD-IDHs. The ScIDH1/ScIDH2 heterodimer is the basic functional unit of NAD-IDH, which is made up of a regulatory subunit ScIDH1 and a catalytic subunit ScIDH2.The mature polypeptides are similar in size (349 and 354 amino acid residues, respectively) and share 42% residue sequence identity. Both subunits are essential for holoenzyme structure

and function [70], and the heterodimer then eventually becomes a heterotetramer and then a heterooctamer [71]. The active site is in ScIDH2, while the allosteric site is in ScIDH1. IDH2 has a catalytic isocitrate/Mg²⁺ and NAD⁺-binding site, whereas IDH1's homologous region is involved in collaborative isocitrate engagement as well as the allosteric activator AMP binding. When citrate (CIT) and AMP which are enzyme activators bind to the allosteric site, the active site undergoes conformational changes at the heterodimer interface, resulting in inducing enzyme activity [72].

- **Eukaryotic NAD-IDHs**

In general, NAD-IDH of mammals, has a more complicated structure and regulatory mechanisms than prokaryotic NAD-IDH. NAD-IDH in mammals is made up of 3 sorts of subunits in a 2 α :1 β :1 γ ratio. The mammalian α subunit is similar in structure to the fungal IDH2 subunit, implying that it acts a enzymatic activity, whilst the β and γ components are structurally similar to the yeast IDH1 subunit, implying that it responsible for regulating enzyme activity. [73]. The weights of the α , β , and γ subunit molecules are approximately 37, 39, and 39 kDa, respectively. The α , and β subunits carry nearly 40% sequence homology, while α , and γ subunits around 42 percent sequence homology, whereas β , and γ subunits have approximately 52 percent

sequence homology. Alpha and beta subunits build the ($\alpha\beta$) heterodimeric structure, and also the α and γ subunits create a second ($\alpha\gamma$) dimer, and finally these 2 heterodimers are joined into a 2($\alpha\beta\gamma$) tetrameric structure and then into a ($\alpha 2\beta\gamma$)² heterooctamer. [63]. As it catalyzes an irreversible and rate-limiting step of the Krebs cycle, it is tightly regulated through substrate availability [citrate, adenosine 5'-diphosphate (ADP), isocitrate (ICT), nicotinamide adenine dinucleotide (NAD⁺), and Mg²⁺/Mn²⁺], product inhibition [reduced form of NAD⁺ (NADH) and α KG], and competitive feedback inhibition (ATP), to avoid unnecessary depletion of ICT and accumulation of α KG. The α subunit is required for ICT binding, the β subunit has structural function by facilitating subunit assembly, and the γ subunit, through binding of citrate and ADP, is implicated in the allosteric regulation of the holoenzyme [74]. Both the $\alpha\beta$ and $\alpha\gamma$ heterodimers' subunits exhibit catalytic properties; nevertheless, only the γ monomer serves a regulatory action through an allosteric regulation, whilst the β monomer has no regulatory involvement but is necessary for optimum performance. Citrate and adenosine diphosphate both positively regulate the $\alpha\gamma$ dimer, but NADH has a negative effect. Furthermore, low amounts of ATP can stimulate these enzymes activity whereas large doses of ATP suppress them. The $\alpha\beta$ heterodimer activity, on the other hand, is suppressed through both NADH and

ATP and cannot be triggered by CIT or ADP[75].

5.3. Physiological metabolic actions of human IDHs

IDH1, IDH2, and IDH3 genes in humans produce 3 variants of the IDH enzyme, all of which do key functions in cell metabolism. IDH1 is present in the matrix of the mitochondria, while IDH2 and IDH3 are distributed in the cytosol and peroxisomal system[76]. Despite their differing locations, IDH1 and IDH2 both are isoenzymes that catalyse the transformation of isocitrate to 2-OG with the help of nicotinamide adenine dinucleotide phosphate (NADP+) as a coenzyme, resulting in NADPH as a product. NADPH in the cytosol perform an essential antioxidant function.[77]. One of peroxisomal IDH1 may be to provide cytosolic NADPH for several NADPH dependent enzymes, such as 3-hydroxy-3-methylglutaryl-CoA reductase, acyl-CoA reductase, and 2,4-dienoyl-CoA reductase. The activity of IDH1 is the only known source for peroxisomal NADPH. Another physiological role of peroxisomal IDH1 is to provide -ketoglutarate, which is required as a cosubstrate for the phytanoyl-CoA-hydroxylase reaction which catalyzes a step in the degradation of phytanoic acid. Several metabolic functions for phytanic acid have been reported such as: white adipocyte differentiation; and activation of uncoupling protein-1 gene transcription and brown adipocyte differentiation [78]. Also, alpha

ketoglutarate-dependent dioxygenases use 2-OG as a cofactor. Ten–eleven translocation (TET) enzymes, used in DNA demethylasesation besides Jumonji-C (JmjC) domain-containing enzymes, which act on histone lysine to remove methyle groups, are the most well-known nuclear 2-OG-dependent dioxygenases[76]. dioxygenases of the cytoplasm include the prolyl hydroxylase domain (PHD)- containing enzymes which have roles in many metabolic processes [79]. IDH3 catalyses the production of 2-OG from isocitrate, but with NAD+ as a coenzyme, and creates NADH, which is employed in the electron transport chain to generate adenosine triphosphate (ATP)[76].

5.4. IDH mutations in cancer

Abnormal metabolism of cancer cells often correlates with mutations in genes encoding for metabolic enzymes, including those involved in the tricarboxylic acid (TCA) cycle and related metabolism. As regard carcinogenesis, the isocitrate dehydrogenase 1 (IDH1) gene is by far the most commonly altered gene of metabolism. Mutant IDH1 and IDH2 genes result in active site replacements in the corresponding enzymes, which have a significant impact on IDH performance, metabolic activity, leading to cancer formation[80]. IDH gene alterations mostly impact the arginine amino acid at position 132 (R132) in IDH1 polypeptide chain, the equivalent arginine 172 (R172) residue in IDH2, or

the arginine 140 (R140) residue in IDH2, rendering these mutational foci [81]. Mutations in the isocitrate dehydrogenase gene IDH1 in cancer were first identified in a whole-exome sequencing study of 22 glioblastomas [82]. Then, it was approved that IDH mutations are prevalent in various types of cancer, including low-grade glioma and secondary glioblastoma (80%), acute myeloid leukaemia (AML; 20%), cholangiocarcinoma (20%), chondrosarcoma (80%), sinonasal undifferentiated carcinoma (49–82%) and angioimmunoblastic T cell lymphoma (32%) [81]. IDH's catalytic domain undergoes structural changes as a result of mutations, leading to the catalysis of a partial reverse reaction, in which α -KG is reduced to (R)-2-hydroxyglutarate [(R)-2HG] [74]. High concentrations of D-2HG negatively affect 2OG oxygenases implicated in epigenetic regulatory mechanisms, such as the Jumonji C domain containing histone lysine demethylases (JmJc KDMs) and the ten eleven translocation (TET) oxygenases, which catalyze removal of methyl groups in certain sites of histone and oxidation of N-methyl cytosine in DNA, respectively. [83]. Competitive inhibition of the JmJc KDMs and the TETs by D-2HG could contribute to the histone and DNA hypermethylation states manifested in IDH variant gliomas [84]. D-2HG's competitive inhibition of the JmJc KDMs and TETs may play a role in the histone and DNA modifications in the form

of hypermethylation states seen in IDH mutant gliomas [84]. These chromatin modifications are thought to prevent malignant transformed cells from differentiating, allowing them to thrive and replicate, encouraging carcinogenesis. [80].

Table (1) Cellular effects of D-2HG:

Effect of D-2HG	Molecular target	consequences	references
inhibition	JmJc KDMs (Jumonji C domain-containing histone lysine demethylase)	Histone methylation, blocked MyoD mediated differentiation, mTOR (the mammalian target of rapamycin) activation	Liu et al. [80], Chowdhury et al. [85], Sulkowski et al. [86]
inhibition	TETs (ten-eleven translocation oxygenases)	DNA methylation	Liu et al. [80], Yeung et al. [83]
inhibition	PHDs (HIF prolyl hydroxylase)	HIF-1 α (hypoxia-inducible factor-	Liu, et al. [80], Sasaki et al.

	domain enzyme)	1a) induction	[87]
inhibition	Collagen prolyl and lysine hydroxylase	Impaired collagen maturation	Sasaki et al. [87]
inhibition	ALKBHs (2OG-dependent AlkB homologue)	Impaired DNA repair	Wang et al. [88], Chene et al. [89]
inhibition	FTO pathway (fat mass and obesity-associated protein)	RNA methylation, suppression of MYCL/C EBPA (CCAAT enhancer binding protein alpha)-associated pathways.	Elkash et al. [90], Su et al. [91]
inhibition	ATP synthase	Mitochondrial respiration, and mTOR signaling reduction	Fuet al. [92]
binding	DMNT1(DNA methyltransferase)	RIP3(receptor-interacting)	Yang et al. [93]

	1)	protein 3) downregulation	
binding	BCAT (branched -chain amino acid transferase) transaminases	Impaired glutamate biosynthesis	McBrayer et. Al. [94]

5.5. IDH mutations in CNS tumors

Adults' most prevalent neurological cancers are gliomas. [95]. The status of the IDH mutation is one of the most important tools of diagnosis of diffuse glioma; accordingly, they can be classified into IDH-wildtype and IDH-mutant type. IDH mutations are more common in low grade gliomas (LGGs) and secondary glioblastomas (GBMs) and are some of the first genetic alterations during the growth of these tumours [76]. IDH polymorphism often take place during glioma formation in its early stages and are insufficient alone for malignant transformation, they indeed, frequently develop subsequent additional genetic disorders such as tumour protein 53 (TP53) alterations, lack of ATP-dependent helicase ATRX, X-linked helicase II (ATRXL), or co-deletion of chromosome region 1p/19q. [96]. IDH mutations are among the most crucial criteria in glioma diagnostic evaluation. IDH alterations,

for instance, are frequently found in diffuse astrocytomas, next come TP53 and ATRX polymorphisms. As regard oligodendrogliomas, IDH abnormalities with 1p/19q co-deletion (with perhaps CIC and FUBP1 mutations) are prevalent. [97]. The presence of an IDH mutation can also help to distinguish between primary and secondary glioblastomas.[98]. Furthermore, because IDH mutations typically result in genome-wide DNA and histone hypermethylation, methylation screening can improve accuracy of classification of glioma even more. As a result, knowing whether the glioma patient has an IDH mutation or not can help physicians to classify the tumors and choose the best treatment options. [96].

5.6. IDHs in other neurological disorders

5.6 1 Neurodegenerative disorders

As a result of their critical role IDH enzymes in oxidation / reduction equilibrium and metabolic activity of the cell, they may be implicated in neurodegeneration[74]. NADPH provided by IDH1 and IDH2 in both cytoplasm and mitochondria, is required for the re-synthesis of glutathione which is neuroprotective against oxidative damage. Lipid peroxidation to proteins, and nucleic acids oxidation with subsequent structural, and functional abnormalities were discovered as a result of lower amounts of reduced glutathione. to be increased in

postmortem brain tissue of PD patients [99]. Similarly, decreased levels of glutathione have been observed in AD and HD. So, glutathione impairment has an important role in the pathogenesis of Parkinson's disease (PD), AD, HD, ALS, and Friedreich's ataxia [100].

5.6 2 Retinal degeneration

Mutations in *IDH3A* have been identified in individuals with inherited retinal diseases (IRDs), which represent a major cause of incurable blindness in children and young adults, and are characterized by progressive degeneration of photoreceptor and/or retinal pigment epithelium cells. Retinitis pigmentosa (RP) is the most common IRD [74].

A diversity of IDH3 polymorphisms linked to RP had been discovered using whole-genome sequencing. Individuals having a homozygous missense mutation in IDH3A codon 304 had the most serious form, exhibiting juvenile encephalopathy along with peripheral and autonomic nervous systems affection, as well as retinitis pigmentosa[101].

6. Other biochemical markers in CNS trauma

6.1. Periostin

Periostin is a protein presents in the extracellular matrix with a molecular weight of 90-kD. It is a neurite outgrowth-promoting factor. It was found

to be increasingly expressed in rat brain tissues after cerebral ischemia or subarachnoid hemorrhage. In patients with TBI, increased serum periostin concentration at admission were strongly relevant to the severity, and short-term mortality, which indicated that periostin serum may be a useful prognostic biomarker in patients with severe TBI [102].

6.2. Glial fibrillary acidic protein (GFAP)

Glial fibrillary acidic protein (GFAP) is an intermediate filament protein presents in the cytoskeleton of astroglia. It has been indicated that GFAP may be used as a marker of traumatic brain injury (TBI) that is released in blood after central nervous system cell injury [103].

6.3. Myelin basic protein (MBP)

Myelin basic protein (MBP) is known as the second most abundant protein in myelin of the central nervous system. Since the 1980s, it has been considered as a biomarker of brain tissue damaged due to either trauma or disease [104].

7. Other genetic markers in CNS tumors

7.1. RELA fusion

Chromothripsis, which includes chromosome 11q13.1, drives C11orf95-RELA fusions. In the nucleus, C11orf95-RELA fusion proteins play a role in

activating members of the nuclear factor- κ B family of regulatory proteins, which mediate the cellular inflammatory reaction and rapidly induce neural stem cells transformation into malignancies such as ependymoma [105].

7.2. TERT (Telomerase reverse transcriptase) promoter mutations

C228T and C250T promoter mutations are the most common TERT mutations. In gliomas, TERT promoter mutations as well as prolonged telomere are predictors of decreased expected lifespan and radiotherapy ineffectiveness. This is commonly noticed in glioblastoma and oligodendroglioma [106].

7.3. O6-methylguanine-DNA methyltransferase methylation (MGMT)

The O6-methylguanine-DNA-methyltransferase (MGMT) gene codes for a DNA repair enzyme that can counteract the impacts of alkylating treatment agents like temozolomide. The introduction of methyl groups through alkylating chemotherapeutic agents causes DNA genotoxicity. So, a tumor with extensive MGMT expression is generally more resistant to chemotherapeutic drugs that target DNA at this site. The gene will be more expressed if the promoter area of the MGMT gene is not methylated, but hypermethylation of the MGMT promoter, will make the gene silenced.

When the MGMT gene is active, the damage can be rapidly repaired. Methylation of the MGMT gene promoter has favorable prognostic and predictive value in glioblastoma patients, but it does not have a diagnostic value in these patients [107].

CONCLUSION

Biochemical and genetic markers such as UCH-L1 and Ng2 are promising tools in diagnosis and classification of CNS trauma and tumors. Also, they can have a role in predicting the outcome of patients with TBI. IDH1 is a genetic marker of great importance in gliomas which are the most common type of CNS tumors. More research is needed on these markers to improve their role in clinical field.

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