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Exploring the Role of Protein Biomarkers in Traumatic Brain Injury: Preclinical and Clinical Highlights

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Abstract

Traumatic Brain Injury (TBI) is a sudden damages to the brain that pose a major public health problem related to morbidity and mortality. The recent epidemiology estimation has crossed over 3 million including military personnel and athletes. Though, the pathology of TBI is wide and not well-known and has been linked to calcium influx, glutamate accumulation, abnormal Amyloid Precursor Protein (APP) expression, oxidative stress, neurotoxicity and neuro-inflammation as well as axonal injury. The axonal injury is a key driver of pathological process following TBI. The studies have reported that the neuro-inflammation after TBI is because of activation of cAMP-PKA inflammatory signalling pathways in neuronal cells damaging CNS. The body fluids like serum, CSF and saliva biomarkers are used to asses axonal injury. Biomarkers are essential diagnostic and prognostic tools plays important role in neurological disorders including TBI. The various substances such as S100-β, tau protein, substances P, BDNF and cystatins D are potential biomarkers targeted in various researches. S100-β protein concentration detection to the preliminary judgment of brain injury, significant rise in the levels of CSF tau protein indicates possible axonal injury. Cystatins D can be novel early biomarkers of TBI. So, in this review the role of different biomarkers of TBI shows the mechanism relationship with clinical outcomes with both clinical as well as preclinical studies.

Keywords: Traumatic brain injury; S100-β; Tau protein; Substances P; BDNF; Cystatins D

Abbreviation

TBI: Traumatic Brain Injury; APP: Amyloid Precursor Protein; PTS: Post-Traumatic Seizure; PTE: Post-Traumatic Epilepsy; TNF: Tumor Necrosis Factor; DISC: Death Inducing Signalling Complex; CTE: Chronic Traumatic Encephalopathy; SP: Substance P; TRP: Transient Receptor Potential; NK1R: Neurokinin 1 Receptor; BDNF: Brain-derived Neurotropic Factor; AD: Alzheimer's Disease; PD: Parkinson Disease; MS: Multiple Sclerosis; HD: Huntington Diseas; CST5: Cystatin D

Introduction

Traumatic Brain Injury (TBI) is defined as a sudden damage to the brain by any force that is a blow or jolt to the head, causes alteration in neurological function. Such forces can include a blunt force trauma, or a piercing or cracking of the skull [1]. TBI refers to permanent or transient neurological dysfunction with broad spectrum of symptoms and disabilities that has resulted from an external force to the brain [2]. Some wide ranging physical and psychological effects may appear immediately after the traumatic event where as some later after few days, a week or afterwards. Globally, TBI has been a major cause for morbidity and mortality particularly during young age. As a major public health issue TBI affecting over 1.7 million Americans annually, with falls, collision incidents and motor vehicle accidents being the leading causes of injury [3].

An estimated 3 million people, including military personnel and athletes, suffer from Traumatic Brain Injury (TBI) annually, accounting for approximately 2.8 million TBI-related emergency hospital visits and deaths in the United States [4]. TBI now has become the leading cause of neurological disability across all age groups. TBI is of two main types that is traumatic injury related damage confined to one area of the brain called as a focal injury, or over a more widespread area termed as a diffuse injury. The primary injury damage is immediate whereas secondary injury damage can occur gradually over the course of hours, days and weeks that are the result of reactive processes occurring after the initial head trauma. The complex and long lasting consequences of TBI can result in serious disorders that involve progressive cognitive deficits, epilepsy, and profound behaviour alteration [5]. TBI has been known as a leading cause of acquired epilepsy or other neurological problems. The brain undergoes distinct electrophysiological changes that can be detected with electro-encephalography. Seizures not only considered being a high morbidity and mortality in the early stages, but also remaining the leading cause of death for several years following TBI. Seizure prophylaxis is commonly employed post-injury with variable success, primarily used for prevention of single occurrence acute Post-Traumatic Seizure (PTS) but has a little efficacy on preventing the recurrent chronic seizures that define post-Traumatic Epilepsy (PTE) [6]. About 4%-53% of TBI patients are still having chronic seizures despite prophylaxis treatment. A hallmark of inflammation is oxidative stress, which can be caused by metabolic dysfunction with numerous potential causes. At the tissue and cellular levels, the pro-oxidative forces of inflammation following an injury can outweigh the capacity of anti-oxidative, protective mechanisms such as super oxide dismutase and glutathione peroxidise. The secondary injury associated with TBI is becoming recognized as equally detrimental as the primary insult because of ensuing morbidity. Indeed, the evolving cell death after the initial impact in TBI accounts for various poorly understood mechanisms such as calcium influx, glutamate accumulation, abnormal Amyloid Precursor Protein (APP) expression, oxidative stress and neurotoxin inflammation along with

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axonal injury. Recently, the axonal injury has been linked with the TBI [7-9]. These biochemical events occur throughout the brain and causes gray and white matter degeneration coupled with motor and cognitive impairments which are key pathological symptoms of TBI survivors [7-10]. The increased glutamate level results in the activation of NR2B that further results in neuronal excitability and death. The activation of extrinsic signalling pathways of apoptosis after TBI is activated by Tumor Necrosis Factor (TNF) and extracellular ligands lead to complex formation that is Death Inducing Signalling Complex (DISC) resulting activation of caspase enzymes which play important role in apoptosis as well as inflammation [11]. The various studies have reported that the neuroinflammation after TBI is because of activation of cAMP-PKA inflammatory signalling pathways in neuronal cells damaging CNS. The mammalian Target of Rapamycin (mTOR) includes the cell growth, differentiation and plasticity by activating kinase enzymes that is mitogen activated protein kinase as well as phosphatidylinositide 3-kinase. The activation of mTOR signalling pathways after TBI leads to autophagy [12].

Proteins as a Biomarker of TBI

Role of S100b in traumatic brain injury

During normal and TBI state, extracellularly administered S100B stimulate neurogenesis and neuronal plasticity as well as improve neuro-modulating functions involved in learning and memory [13]. S100B performs a dual function that is at low concentration it is beneficial and at higher concentration the effects are harmful [14,15]. Rapidly increasing, extracellular levels of S100B shown to result in cell death and neuronal dysfunction because of an inflammatory response that activates astrocytes and microglia along with extracellular elevated calcium level and nitric oxide activation show harmful effects shown in Figure 1 [13-16]. The BBB of the patient suffering from TBI gets disrupted causing the leakage of proteins from CSF following cerebral deterioration and formation of edema [17]. The albumin ratio between CSF: serum (QA) is sometimes used to detect the degree of disruption of BBB [18]. Some authors claim that through the disrupted BBB, S100B is released into the serum. The concentration of S100B in the CSF could be up to 100 times higher than in serum [19].

Role of Tau protein in traumatic brain injury

Studies have purposed that Traumatic Brain Injury (TBI) induced loss of microtubules density has been found to be associated with a 45% decreases in the levels of microtubules associated proteins in previous immunochemical studies. This suggest that microtubule associated protein may severe as potential biomarkers for TBI [20]. Therefore, various study investigated the potential of using Tau protein as a biomarker of TBI [21]. Tau protein, a new marker is a microtubule associated protein that primarily localized in the axonal compartment of neurons. Functionally, tau protein binds to axonal microtubules, resulting in the formation of axonal microtubule bundles. These bundles become important structural elements in the axonal cytoskeleton and are critical elements in the axoplasmic flow of proteins between the nerve terminal and the neuronal cell body [22]. The tau protein may provide a useful marker of CNS injuries. Human brain tau protein has 6 isoforms, with a molecular mass of 48-67 KDa upon sodium dodecyl sulfate polyacrylamide gel electrophoresis [23]. Following axonal injury, tau becomes modified primarily by hyper-phosphorylation of various amino acid residue and cleavage into smaller fragments. These post trauma products can leak into the cerebrospinal fluid or blood stream and become candidate biomarkers of CNS injury. Increased levels of CSF tau protein are a possible sign of axonal injury and have been reported in AD [24]. While tau protein can be measured in the CSF of both healthy and pathological cases, its dissection in the serum of healthy individuals is difficult. However, tau protein becomes detectable following TBI, since the damage to axons results in its release from CNS neurons [25].

Zemlan et al. [26] has studied patients with severe head injury whose Glasgow Coma Scale (GCS) was no greater than 8; they reported that tau protein level in the CSF was significantly increased and that initial tau protein levels in patients with poor GCS scores. They concluded that measurement of CSF tau protein level might be beneficial in patients

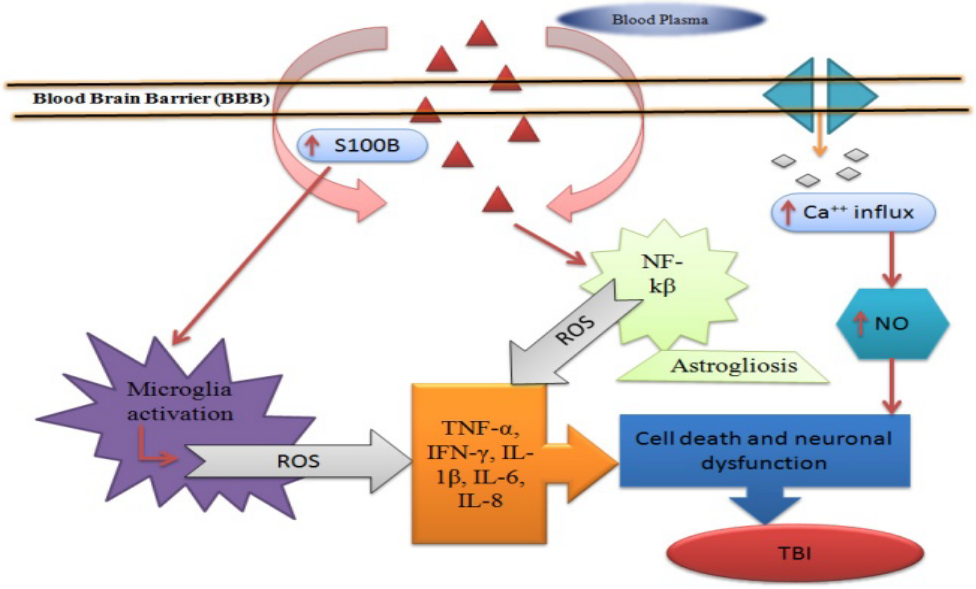

Figure 1: Role of S100b in traumatic brain injury.

with severe head injury [26]. In clinical field, serum tau protein has the advantage that can be measured by less invasive method compared with those used to measure CSF tau proteins. Numerous studies revealed that tau protein levels are higher in CSF in severe TBI patients, but only a limited no. of studies has investigated the serum tau protein levels [27].

Bulut et al. [20] has studied that the objectives of this study was to investigate the diagnostic value of serum tau protein in determining the severity of TBI in proteins with mild TBI (mTBI) and higher risk patients. Serum tau protein levels of 60 proteins and 20 healthy volunteers, who served as a control group, were measured. The mean age of the 60 patients (45 male and 15 female) was 32.5 years (range 15-66 years). The mean Glasgow Coma Scale (GCS) score was 14 ± 0.6. Serum tau level of patients (188 ± 210 pg/mL), compared with those of control (86 ± 48 pg/mL), were relatively higher. However, differences were not statically significant (p=0.445). These studies were found that the tau protein level in increases after onset of mTBI, and that this increases is significant in patients at high risk for mTBI. If the diagnostic sensitivity of the serum tau protein in detecting traumatic brain injury follows mTBI is to be definitely established [20]. Military personnel who reported three or more TBI showed high total tau protein concentration in plasma, in some cases long after the injuries had occurred, an observational study indicated. These finding suggest that accumulations of the plasma biomarker, tau may contributed to chronic neurological symptoms following TBI. This suggest there are additionally deposited of hyper phosphorylated tau are well known features of Alzheimers disease and have also been found in the condition known as chronic traumatic encephalopathy or CTE [28].

Wang et al. [27] performed the experiment in serum tau protein as a potential biomarker in the TBI the conclusion of those articles. The mean serum tau protein levels were significantly higher in the severe TBI group compared with those in the mild and moderate group. In addition, positive correlation was observed between high serum tau protein levels and poor outcome in TBI patients. Therefore, the finding of the current study suggest that serum tau protein may serve as a potential biomarkers used to evaluated the severity and predict the outcome of TBI patients [27-29].

Role of substance P in traumatic brain injury

Substance P (SP) is a member of the tachykinin family of neuropeptides, which are widely distributed throughout the Central Nervous system (CNS) [30]. SP is initially released by the activation of the membrane of the transient Receptor Potential Family (TRP), TRPV1 and TRPA1. SP are actively involved in inflammatory responses mediated by activation of its preferred Neurokinin 1 Receptor (NK1R) which is widely distributed throughout the CNS [31]. Brain injury increases NK1R expression in neuron and astrocytes and SP acting through this receptor, leads to activation of astrocytes. Reactive astrocytes proliferate and produce several soluble pro-inflammatory mediators, such as cytokines, prostaglandins and thromboxane derivatives [32].

In addition, both SP and NK1R are expressed in microglia cells, which are involved in initiation and progression of immune responses with in CNS [33]. Stimulation of microglia by SP initiates activation of nuclear factor KB (NF-KB) a transcriptional activator involved in expression of pro-inflammatory cytokines. In fact microglia produces Interleukin (IL)-1 in response to SP. Apart from microglia, other brain cells can be activated by SP. In this sense, it has been shown that SP interacts with NK1R receptor present on human neuronal cell line NT2N, inducing the expression of the potent chemokine macrophage inflammatory protein 1 [34]. SP can activate the transcription factors NF-KB and p38 mitogen activated protein kinase in astrocytes cell line, leading to the production of the pro-inflammatory cytokine IL-1, IL-6 and IL-8 shown in Figure 2 [35].

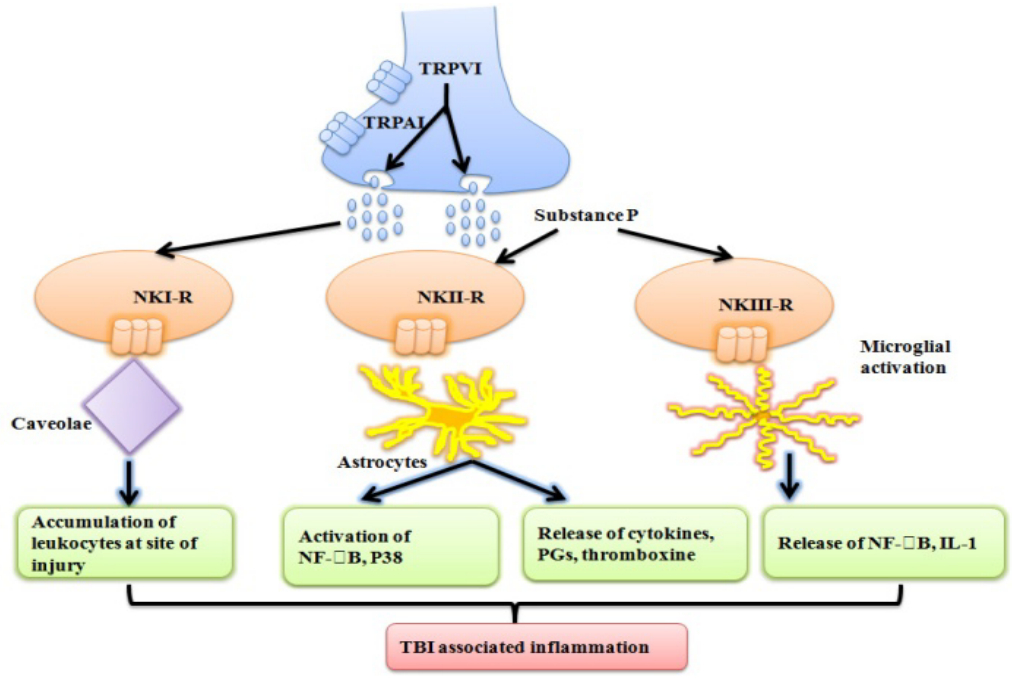

Figure 2: Role of substance P in traumatic brain injury.

Finally, SP can promote leukocyte chemotaxis through NK1R expressed in many inflammatory and immune cell and in endothelial cells (mainly in caveolae) leading to the extravasations, migration and subsequent accumulation of leukocytes at sites of injury. All these data suggest that SP is actively involved in inflammatory process following brain injury. Although SP increases after damage can be beneficial in fighting host infections association with TBI [36]. It may also play an important role in inflammatory immune response in the CNS, which may be fatal for patients with TBI. Serum SP levels were associates with injury severity and mortality in patients with severe TBI and whether these levels would be clinically useful in predicting mortality in patients [37].

Extensive research has shown that levels of SP rise acutely following TBI in both preclinical animal models and in human tissue. Virtually, all blood vessels of the body are surrounded by sensory nerve fibers that contain SP [38]. In TBI it has been demonstrated that perivascular SP immunoreactivity increases in preclinical model, irrespective of injury model and also in human [37-41]. It appears that SP is released early following TBI, with increases noted in the plasma at 30 min following TBI in rodent [39]. Furthermore this release of SP appears to depend on the magnitude of the insult, with a graded increase in SP immune reactivity seen with increasing severity of injury [41]. Moreover, it has been shown that attenuating SP activity following TBI is beneficial to outcome. The first demonstration of neurogenic inflammation in TBI showed that depletion of sensory neuropeptides by pre-treatment with capsaicin results in the attenuation of post traumatic BBB permeability edema formation and improved functional outcome [42].

Later studies, specially targeted SP by administrating an NKL antagonist showed beneficial effects in both male and female rats with a significant attenuation of post traumatic BBB permeability and a resultant significant reduction of edema formation with improvement in motor and cognitive outcome [37-42]. Clinically, the serum SP levels in patients with severe TBI have been demonstrated. The non-surviving TBI patients with showed higher serum SP levels than survivors, that serum SP levels were associated with severity and mortality and that serum SP levels could be used as biomarker to predict mortality in patients with severe TBI [43,44]. These results open the possibility that NK1K antagonists may be useful for the treatment of severe TBI.

Role of brain-derived neurotrophic factor (BDNF) in TBI

Brain Nerve growth factor was discovered in the early 1950s due to its trophic effects on sensory and sympathetic neurons [45]. In 1982, Brain-Derived Neurotropic Factor (BDNF) the second member of the "neurotrophic" family of neurotrophic factors, was shown in promote survival of a sub-population of dorsal neuron of ganglion neurons and subsequently purified from pig brain [46]. Brain-Derived Neurotropic Factor (BDNF) plays an important role in neuronal survival and growth serves as neurotransmitter modulator and participates in the neuronal plasticity which is essential for the learning and memory. BDNF is a neurotrophin that protect neurons against glutamate excitotoxicity [47]. It is widely expressed in the CNS, gut and other tissues and regulates glucose and energy metabolism and prevents exhaustion of beta cells.

Moreover, decreased level of BDNF is associated with neurological diseases with neuronal loss, such as Alzheimer's Disease (AD), Parkinson Disease (PD), Multiple Sclerosis (MS) and Huntington Disease (HD) [48]. BDNF plays an important role following TBI. In response to TBI, the mRNA expression level of BDNF is transiently and significantly increased [49]. Studies have reported that within hours of post injury, the expression level of BDNF mRNA is significantly up regulated in the injured cortex and in the hippocampus. The BDNF level declines at 24 hours post injury and is no longer significant at 36 hours of post injury [50]. Following injury the mRNA expression level of Tropomyosin receptor Kinase B (TrKB) receptor is also transiently up regulated in the hippocampus and dentate gyrus [51]. This transient of BDNF acts as an endogenous neuroprotective response attempting to attenuate secondary cell damage following TBI shown in Figure 3 [52]. The importance of the BDNF/TrKB signalling pathway in regulating CNS function has led to many studies exploring the therapeutic potential of BDNF/TrKB for various neurological diseases including TBI. The therapeutic potential of BDNF is restricted due to its short half-life (<10 min) and inabilility to cross the Brain Blood Barrier (BBB) because of the large size (27KDa) [53]. Thus far, directly application of BDNF for TBI has not been efficacious in experimental TBI studies.

However, limited studies have been shown when delivered indirectly BDNF can significantly improve functional recovery of injured animals. Intravenous injection of nanoparticle bounded BDNF, increased BDNF levels were found in the brain and animal had improved neurological and cognitive function following a weight drop injury in mice [54]. Clinically studies on BDNF levels in TBI patients have been scarce and the results have been controversial thus the role in TBI remains unclear. On the one hand, an association between TBI patients out come and BDNF level in serum or cerebrospinal fluid have not been found on the other hand an association between poor TBI patients' outcome and low BDNF levels in serum as well as high BDNF levels in cerebrospinal fluid have been found [55]. A prospective observational clinical study of children with TBI and BDNF up regulation in the CSF after injury was associated with better neurological outcomes [56]. Based on this finding BDNF expression is a useful biomarker of brain damage following severe TBI [57].

Role of Cystatin D in TBI

The proteins containing cystatins-like sequences surround the cystatins family. Some of the members are active cysteine protease inhibitors, while others have lost or perhaps never acquired this inhibitory activity. There are three inhibitory families in the super family including the Cystatins (type 1 and 2) and the kininogens. The cystatin locus on chromosome 20 contains the majority of the type 2 cystatin genes and pseudogenes, located in the cystatin locus. The encoded

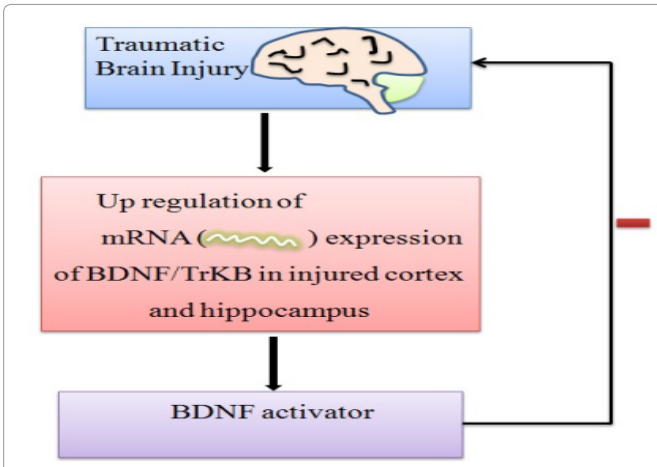

Figure 3: Role of Brain-Derived Neurotrophic Factor (BDNF) in traumatic brain injury.

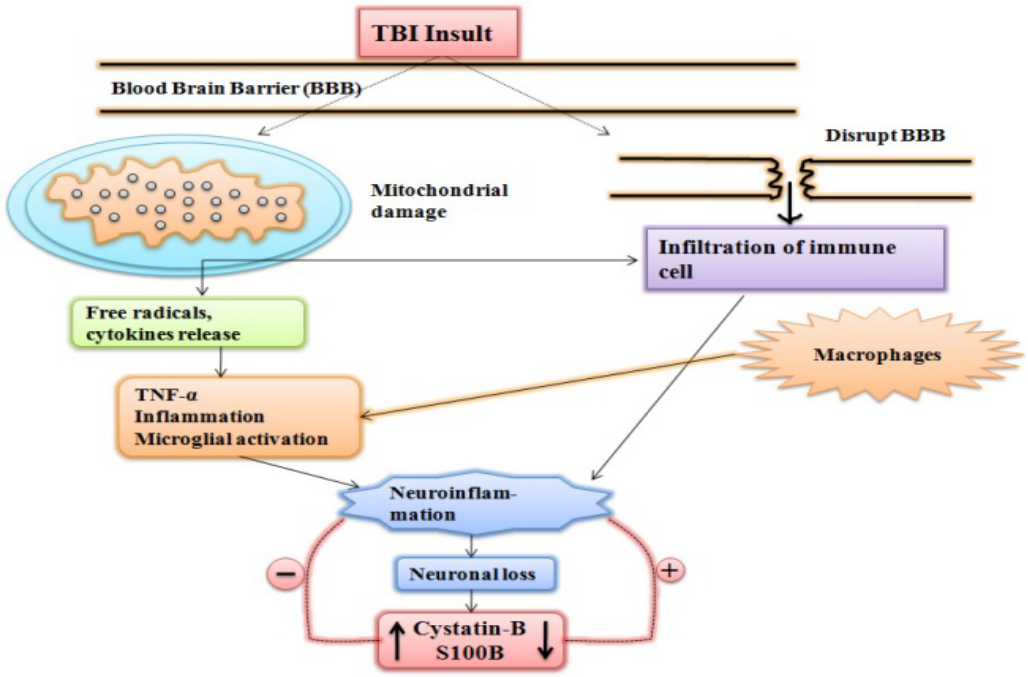

Figure 4: Role Cystatin D in traumatic brain injury.

protein may play a protective role against proteinases present in the oral cavity [58]. Cystatin D (CST5) is an inhibitor of lysosomal and secreted cysteine proteases 32. It was originally purified from saliva 33 and inhibits proliferation, migration and invasion of colon carcinoma cells indicating a tumor suppressor activity that is unrelated to the protease activity 34 [59]. Transcription analysis has also showed that cystatin D might alter gene expression, including that gene encoding transcription factors such as RUNX1, RUNX2 and MEF2C inHCY116 cell 34. None of previous protein biomarker has been successfully used in the clinical setting for diagnosis and prognosis of TBI patients [60]. So, CST5 was identified as a potential biomarker to assess the severity of TBI and its expression at very early time points makes CTS5 an ideal biomarker for a Point of Care (PoC) device shown in Figure 4 [61]. CST5 has demonstrated the ability to differentiate between severely TBI patients from all other cohort those with either mild or no brain injury within the first hour highlighting it as a new biomarker. Therefore, CST5 can be novel early biomarkers of TBI [60].

Conclusion and Future Perspective

TBI is a complex dynamic process that initiates a multitude of cascades of pathological cellular pathways. Pathology of TBI is wide and so far has been linked to calcium influx, glutamate accumulation, abnormal APP expression, oxidative stress, neurotoxicity and neuro-inflammation along with axonal injury. The various substances such as S100-B, tau protein, substances P, BDNF and Cystatins D are potential biomarkers targeted in clinical case studies. These proteins concentration may results in neurogenesis as well as activation of microglial cells, astrocytes, Ca^{2+} influx mechanism, leads to the release of NO and other inflammatory cytokines playing role in pathology of TBI. Although, the research in TBI has increased exponentially from last 20 years, there is a greater need to explore biomarkers in population. These biomarkers could be potentially facilitating diagnosis and treatment of TBI. The role of different biomarkers of TBI shows the mechanism relationship between both clinical as well as preclinical studies. Each biomarkers of this review could be carefully considered for future application in research.

Conflict of Interest

The authors declare that there is no conflict of interest.

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