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Narrative Review Open Access

Diagnostic salivary biomarkers in traumatic brain injury: narrative review

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Abstract

Traumatic brain injury (TBI) is a common cause of disability and mortality worldwide. TBI is an acquired brain injury that may be open (penetrating) or closed (non-penetrating) and is be categorized as mild, moderate, or severe, depending on the clinical presentation. Accurate diagnosis at the earliest stages can significantly affect patient discomfort, prognosis, therapeutic intervention, survival rates and recurrence. Whereas traditional CT and MRI techniques for diagnosis are dominant in clinical situations, a promising direction for clinical diagnosis is the use of fluid biomarkers like blood, CSF, urine, and saliva. Fluid biomarkers that may track these injuries and inflammatory processes have been explored for their potential to provide objective measures in TBI assessment. At present, there are limited clinical guidelines available regarding the use of fluid biomarkers in TBI.

In recent years, saliva has received significant attention as a biomarker for TBI in clinical practice due to the non-invasive accessibility, cost-effective collection, and consistent relationship with serum. This review examines the utility of saliva biomarkers such as S100B, noncoding RNAs (ncRNAs), extracellular vesicles (EVs), miRNAs levels, microtubule-associated protein tau, alpha-amylase, cortisol, and oxidative stress in TBI.

The study highlights the current state of salivary diagnostics, future aspirations, and their potential as the preferred route of TBI detection. The newly developed techniques for salivary analysis of these molecules may help to improve outcomes for TBI through rapid detection current unavailable with serum samples. Future studies via salivary biomarkers will help establish consistent strategies for early diagnosis of TBI and improve treatment outcomes of TBI patients.

Keywords: Saliva, Traumatic Brain Injury, Head trauma, Biomarker.

Introduction

Globally, traumatic brain injury (TBI), is the greatest single contributor to disability and death of all trauma-related injuries. The accurate identification and diagnosis of TBI is the first step toward providing appropriate clinical care. However, accurate clinical identification of patients with TBI is complicated by variations in the criteria used for diagnosis.[1] There are no conclusive biologic tools to detect TBI or to track brain recovery, diagnosis and management largely consist of patient-reported symptoms and subjective clinical assessment. There is increasing interest in employing saliva samples as a source of biomarkers for TBI in clinical practice due to the non-invasive accessibility, cost-effective collection, and consistent relationship with serum.[2] New methods for saliva handling, analysis, and biomarker discovery offer strong promise for the components of saliva as biomarkers for TBI. These methodological advances have identified

new candidate biomarkers of TBI, and present the feasibility of their use for diagnostics and prognostics of TBI.[3] If successfully validated, saliva biomarkers of TBI will break new ground by improving clinical management of TBI and advancing TBI treatments.

Traumatic brain injury

Damage to the brain after trauma is referred to as traumatic brain injury (TBI). TBI may be blunt, non-penetrating, penetrating, or the result of a blast. The resulting neuropathology consists of a primary injury that is a direct consequence of the traumatic insult and a secondary injury that results from a cascade of molecular and cellular events triggered by the primary injury and that leads to cell death, axonal injury, and inflammation. The annual incidence of TBI has been estimated to be 27 to 69 million, worldwide. Many survivors live with significant disabilities, resulting in a major socioeconomic burden. The symptoms of a TBI can be mild, moderate, or severe, depending on the extent of damage to the brain. Mild trauma may induce brief changes in mental state or consciousness. Severe trauma may induce prolonged periods of unconsciousness, coma, or even death.[4,5]

A computed tomography scan (CT scan) is the gold standard for the radiological assessment of a TBI patient. A CT scan is easy to perform and an excellent test for detecting the presence of blood and fractures, critical markers in medical trauma cases. Plain x-rays of the skull are recommended by some as a way to evaluate patients with only mild neurological dysfunction. Magnetic resonance imaging (MRI) is not commonly used for acute head injury since it takes longer to perform an MRI than a CT. Because it is difficult to transport an acutely injured patient from the emergency room to an MRI scanner, the use of MRI is impractical. Besides these accurate tools, the assessment of biomarkers in biofluids like blood, CSF, urine, and saliva have received attention and present some valid results.[6]

Despite progress in preventive and therapeutic strategies, delay in TBI diagnosis remains one of the major causes of high morbidity and mortality. The prevalence of TBI is rising and leads to an increase in the burden of the socio-economic and health system, so rapid and accurate diagnosis of TBI is necessary.

Saliva as a source of biomarkers

Human saliva is a clear and slightly acidic heterogeneous biofluid (pH 6.0 to 7.0) composed of water (99%), proteins (0.3%), and minerals (0.2%). On average, individual saliva secretion can vary from 0.3 to 0.7 ml of saliva per minute, producing a range of 1 to 1.5 liters per day. Saliva assists as in a variety of functions including tasting, swallowing, and digestion of food. Saliva also plays a role in fat deposition and serves as a protective barrier against pathogens.[7]

Saliva is produced in the salivary glands by acinous cells, and released into the oral cavity through a series of ducts. The parotid, submandibular, and sublingual, create more than 90% of total saliva, with the smaller glands, (lip, buccal, lingual, and palate) providing the rest.[7,8]

Saliva is collected and analyzed as unstimulated whole saliva, unstimulated saliva from specific glandular pairs (e.g. parotid or submandibular and sublingual pairs), or stimulated saliva from specific pairs of glands. Whole saliva that is present in the oral cavity for 24-hour periods is characterized as “unstimulated whole saliva” and is normally associated with precise clinical conditions when compared to stimulated saliva, because the substances used to prime the flow may affect saliva composition. Unstimulated saliva is collected from an individual’s mouth by passively allowing it to flow into a container, or saliva is held in the mouth for a specific period and spit into a container. This method of collecting is the "gold standard" for obtaining many saliva components.[9]

Saliva contains several growth factors including EGF, FGF, NGF, and TGF-α, that are essential for the regeneration of the oral and esophageal mucosa. Some antibacterial and antifungal components are also found in saliva, such as lysozyme, immunoglobulins, and lactoferrin, that prevent the progression of bacterial infection and tooth decay. An important proteolytic enzyme, α-amylase, is secreted by saliva. Some of these components may serve as diagnostic biomarkers, that can be accurately analyzed using specific and sensitive immunological and biochemical techniques such as RIA, ELISA, and chromatography.[10]

Recent technological advances in the processing and evaluation of salivary components have yielded reliable results increasing the characterization of this biological

resource as a relatively safer, cheaper, and less invasive measure than traditional samples such as blood. Collecting blood carries potential risks to individuals, including transient discomfort, bruising, infection at the site of the vessel, and anemia (if large volumes or vulnerable individuals are required). Saliva samples can have several advantages over blood for routine TBI testing, including their safe and easy collection.[11]

According to the National Institutes of Health (NIH), a biomarker is an objective indicator of the measurement and evaluation of natural biological processes, pathogenic processes, or drug responses to therapeutic interventions whose concentration, structure, function, or inactivity are correlated with the onset, progression, or even regression of a particular disorder or as a result of the body's response to it. Thus, biomarkers act as valuable and attractive tools in the diagnosis, risk assessment, diagnosis, prognosis, and monitoring of the disease.[12]

The use of salivary biomarkers as new tools for the diagnosis of TBI are emerging. Recent studies that have examined the use of biomarkers in the saliva, including S100B, noncoding RNAs (ncRNAs), extracellular vesicles (EVs), miRNAs levels, microtubule-associated protein tau, alpha-amylase, cortisol, and oxidative stress are described below.

Salivary S100B

Protein S100B is a member of the S100 protein family and comprises the largest subset of Ca^{2+} EF-hand-binding proteins. S100B is mainly synthesized by astrocytes in the human brain and secreted from glial cells into the extracellular space, where it exhibits cytokine-like functions, and modulates long-term synaptic plasticity.[13]

The structure of the S100B protein, provided by the X-Ray technique, is an octagon of four homodimeric units arranged as two tetramers in a tight array. In Escherichia coli, several other recombinant types of S100B, including tetrameric, hexameric, and octameric, have been identified. Related studies have shown that the binding of the S100B tetrameric structure to RAGE receptors (recipients of advanced glycation end products) occurs with greater affinity than its dimeric structure. In this regard, Tetramer S100B activates cell growth more strongly than dimer S100B and increases cell survival.[14]

Although the highest amount of S100B has been measured in glial cells of the central nervous system its persistence and extracellular function lead to the detection of this protein in non-CNS cells such as skeletal myofibers, myoblasts, adipose tissue, and melanocytes, and other tissues. Due to the presence of this biomarker in the systemic circulation, it has the ability to be present, examined, and measured in biological body fluids such as blood, urine, saliva, and amniotic fluid.[15]

The S100B levels in body fluids, including blood and saliva increase after TBI, heavy exercise, and neurological diseases. Therefore, an increase in the level of S100B can be considered as a sign of nerve cell damage. A study of 15 adult patients with suspected TBI (mean age= 47 years, range 18–79) and 15 control subjects (mean age= 33 years, range 23–53) found salivary S100B levels were 3.9 fold higher than blood S100B, regardless of the presence of pathology. The salivary level of this protein was as accurate in differentiating TBI patients from control subjects as serum levels. These preliminary results suggest that salivary S100B measurements could be a reliable alternative to serum S100B in the diagnosis of TBI. Further studies with a large sample size are needed to confirm these findings.[11]

Early detection of blood-brain barrier damage and intracranial hemorrhage within minutes is critical to prevent permanent brain disability after TBI. This detection requires measurement of the S100B protein level with some 100% validity. To measure serum levels, plasma samples are separated by centrifugation within 2 hours of collection, the serum is collected and stored at -70 ° C. Serum levels of S100B protein are determined using an ELISA kit. The same kit can be used to directly detect the protein S100B in saliva (without centrifugation, separation, or storage), the time saved is an important benefit of saliva over plasma. Utilizing salivary samples of this protein in cases of TBI has other potential benefits including non-invasive sampling and lack of infection risk.[16]

Salivary noncoding RNAs (ncRNAs)

Non-coding RNA (ncRNA), discovered in 1950, is RNA that is not translated into a protein. Instead, ncRNAs act as guides directing proteins to specific target sites and play

important roles in differentiation, epigenetic regulation, transcription regulation, and post-transcription regulation.

ncRNAs are widely distributed in various tissues, although some ncRNAs have tissue-specific expression.

Intracellular localization of ncRNAs can be found in a wide range of intracellular components such as the cytoplasm, nucleus, or both. The number of ncRNAs in the human genome is unknown; however, recent transcriptomic and bioinformatics studies suggest that this classification includes thousands of sequences. ncRNAs are classified by length [Figure-1]. These functionally important types of ncRNAs are including tRNA, rRNA, microRNAs, siRNAs, piRNAs, snoRNAs, snRNAs, exRNAs, and scaRNAs. There are several epigenetic changes, including DNA methylation and histone modification, and changes in ncRNA levels that may occur as a result of TBI.[17-19]

One of the most important features of ncRNAs is their high stability, especially when exposed to exosomes and apoptotic bodies. ncRNA is detectable in body fluids like blood and saliva suggesting their potential usage as biomarkers for screening, diagnosis, prognosis, and response to treatment and evaluation of treatment.[20]

In the UK Men's Rugby Union, physicians used ncRNAs as salivary biomarkers for the assessment of head injuries in athletes.[21] A study on the hippocampus of rats showed that the expression level of circRNA, which is a type of ncRNA, changed significantly after TBI. These results suggest that altered circRNA expression patterns in the rat hippocampus after TBI may play important roles in post-TBI physiological and pathological processes. These findings may provide not only a new direction for studying the molecular mechanisms underlying TBI but also a new possibility for the treatment of TBI by modulating circRNAs.[22] In another study, RNA sequencing was performed on 505 saliva samples obtained longitudinally from 112 individuals (8–24-years-old) with TBI. ncRNA biomarkers show promise for tracking recovery from TBI, and for predicting who will have prolonged symptoms. Salivary ncRNA levels represent a non-invasive and biological measurement that could aid in the accurate, early diagnosis of TBI, improving clinical outcomes for patients.[23]

Salivary MicroRNAs

MicroRNAs (miRNAs) are small, endogenous, non-coding molecules with a length of 19-42 nucleotides that were discovered in 1993. miRNA genes can be very different in position in the genome. We have 2 separate classes of miRNAs: One group is encoded by protein-encoding transcription introns and the next group is encoded by exons. miRNAs are evolutionary RNAs that act as regulators of post-transcriptional expression and affect the translation of proteins throughout the body. miRNA primarily inhibits or modifies the production of a protein produced by binding to complementary target sequences in mRNA and interfering with translation machines. In addition to suppressing translation, the binding of miRNA to mRNA causes the uptake and association of mRNA decay factors and leads to mRNA instability, degradation, and consequently reduced expression levels.[24,25]

miRNA is found in every human tissue and biofluid. The CNS contains the highest concentration and highest diversity of miRNAs. It is estimated that 70% of all miRNAs are expressed in the brain, spinal cord, or peripheral nerves. These molecules are resistant to RNAse degradation, have the ability to cross the BBB, and are transported through protected extracellular space in exosomes and microvesicles, allowing them to be easily measured in biofluids, including serum, cerebrospinal fluid, and saliva.[26]

Due to their abundance, stability in pH fluctuations, resistance to enzymatic degradation, and their key role in transcriptional regulation, miRNAs are potentially ideal biomarkers for the diagnosis, identification, and classification of cancers and other diseases, including neurodegenerative diseases, diabetes, and TBI patients. These molecules are critical to the function of neurons and regulate gene expression in response to brain damage.[27]

Saliva can receive exosomal miRNAs directly from the cranial nerves in the mouth and throat, therefore responds faster than blood miRNAs that must pass through the BBB. Salivary miRNAs, on the other hand, reflect the secondary neuroplasticity response, which explains their high diagnostic accuracy.[27]

Most studies have examined miRNA levels in peripheral blood, and a few recent studies have identified saliva as a new biofluid. Hicks et al., suggest that these miRNAs could predict TBI status in individuals, and salivary miRNA levels were associated with the severity of mental symptoms.[28] In another study, saliva samples were collected at multiple time points, both pre-and post-fight from 50 amateurs mixed martial arts fighters. A subset of salivary miRNAs showed robust utility at predicting TBI likelihood and demonstrated quantitative associations with head impacts as well as cognitive and balance measures.[29]

Salivary Extracellular vesicles

Extracellular vesicles (EVs) are bilayer vesicles of phospholipid membranes that are secreted by different cells. These particles contain biological molecules such as mRNA, miRNA, DNA, proteins, and lipids. EVs were initially described as a way for homeostasis and biological waste disposal. Today, the important and influential role of EVs in intracellular communication is very evident not only in normal functions but also in disease conditions such as cancer, autoimmune diseases, cardiovascular and respiratory failure as well as trauma, and tissue damage.[30]

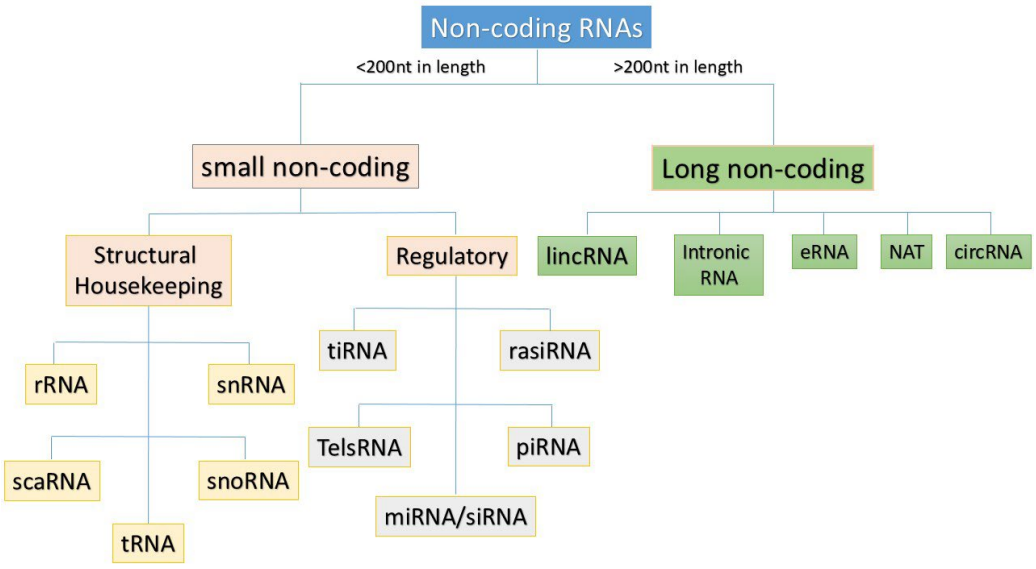

Figure 1. Classification of non-coding RNAs

EVs may affect bone marrow stromal regeneration, target cell function, angiogenesis, progression, and metastasis of blood malignancies by inducing gene expression in target cells. EVs mediate communication between cells by affecting target cells and regulate physiological and pathological processes.[31,32]

The recent classification of EVs is based on biophysical characteristics such as size, cellular origin, molecular content, and biogenesis. According to these criteria, EVs are categories to exosomes, microvesicles, and apoptotic bodies, and oncosomes.[33]

These EVs contain the genomic and proteomic content of the stem cell, can be used as potential biomarkers, and are available from biological fluids such as serum, urine, and saliva.[34]

Accurate and fast diagnosis of TBI is vital for patients. In recent years, there has been a growing interest in EVs that contain a variety of biomarkers and as an indicator of the status of target cells. In fact, EVs can be considered as a biopsy platform in the field of early diagnosis and treatment. Studies have shown that EVs released by damaged cells to biological fluids can be used as potential biomarkers to diagnose and evaluate the severity of TBI.

EVs move between the brain and the oral cavity and can be isolated from salivary specimens as a non-invasive TBI biomarker that may highlight severe changes or neuropathology.[35,36]

In a study of 19 subjects with 7 healthy controls, 6 patients diagnosed with concussion injury from an outpatient concussion clinic, and 6 patients with TBI who received treatment in the emergency department. Real-

time PCR analysis of salivary extracellular vesicles in participants showed significant changes in the expression of 15 inflammation-related genes. These findings indicate that inflammation biomarkers can be used for the diagnosis of TBI and the evaluation of disease severity.[35] Also, findings from another study conducted on 8 mixed martial arts (MMA) fighters and 7 controls, suggest that salivary EVs may be used as a biomarker in the acute period following TBI to identify the severity of the injury severity and to elucidate pathophysiological processes involved in TBI.[37] Considering the consistent results in several studies, as well as the non-invasiveness and relatively fast sampling from saliva, it can be mentioned that salivary EVs can be used as a diagnostic biomarker to detect TBI.

Microtubule-associated protein tau

Microtubule-associated protein tau (MAPT) is a multifunctional intracellular protein that belongs to the MT-associated protein family. MAPT is predominantly expressed in neurons and is highly enriched in the axonal regions. In the human brain, MAPT presents high immunoreactivity in non-myelinated axons of cortical interneurons located in the grey matter. MAPT has been recognized as a biomarker of axonal disruption and the release of MAPT into blood and CSF serves as an indication of neurotrauma. Elevated levels of MAPT have been observed in mild and severe TBI.[38-40]

Although the role of MAPT as a biomarker in the case of TBI has been investigated by examination of CSF and blood obtained during a forensic autopsy, less is known concerning MAPT liberation and occurrence in other biofluids like saliva. MAPT levels were significantly elevated in saliva of those in a study group that was selected following neuropathological examination compared to the control group.[42] The elevated MAPT concentration levels in saliva were predictive of the axonal injury even in cases where the head injury was not considered to be the direct cause of death and thus were undiagnosed and omitted during the regular forensic autopsy.[43] In another study, the elevated salivary MAPT concentration levels using ELISA were recorded in cases of TBI in population-based autopsy screening, as a biomarker for axonal injury.[42]

MAPT release in saliva is provided by two distinct mechanisms. Secretion of saliva is mediated by the principal salivary glands such as sublingual, submandibular, and parotid that are innervated through parasympathetic components of the cranial nerves VII (facial), IX (glossopharyngeal), and their associated submandibular and otic ganglia.[44] This close anatomical relation of salivary glands with the nervous system supports the hypothesis that MAPT is directly released from their innervation. Alternatively, MAPT could pass from systemic blood circulation into the saliva through the blood-saliva barrier which exchanges kinetics of molecules that remain largely unaltered in the event of head trauma.[45] Thus, elevated concentration levels of MAPT in saliva should be considered as a potential biomarker for TBI in postmortem examination.

Salivary alpha-amylase

Salivary alpha-amylase (sAA) is a digestive enzyme produced by salivary acinar cells that break down alpha-1 to 4 glycosidic bonds in starch and related carbohydrates to produce glucose, maltose, maltotriose, and dextrin. During the past years, sAA has presented as a valid and reliable biomarker for autonomic nervous system (ANS) activity in stress research.[46] It has also been observed as a non-invasive biomarker for sympathetic nervous system (SNS) activity.

In a prospective cohort study, sAA was elevated in children 13-15 yrs old after TBI and the levels of sAA predicted specific post-traumatic stress symptoms in children.[47] In another study, Salivary alpha-amylase increased in response to trauma reflection in 20 women (mean age 23.6 +/- 5.8 years) with a history of trauma exposure. Salivary alpha-amylase might serve as a more reliable marker of trauma-related reactivity to negative affective information, and also as a marker of hypervigilance in the absence of threatening information.[48] As sAA is one of the most abundant components of saliva, it may prove to be a reliable biomarker in the diagnosis of TBI and stress.

Salivary cortisol

Cortisol is a low molecular weight lipid steroid hormone that is produced and secreted by the cortical part of the

adrenal glands. Hormone production is regulated by the hypothalamus in the brain and pituitary gland (HPA axis). Most cortisol in the blood (up to 95%) is protein-bound and only a small percentage is "free" and biologically active. Free cortisol is present in saliva, too. Studies have shown that salivary cortisol can be kept stable for at least 5-7 days at Room temperature.[49,50]

In a study, relative to a healthy non-injured comparison group, TBI-injured children (ages 8-12 years) had higher cortisol levels following vehicular accidents.[47] In another study, the most common abnormalities included cortisol elevation in 34 adult TBI patients, following weaning from mechanical ventilation.[51] Conversely, another study salivary cortisol was not increased in response to the trauma reminder in 20 women with a history of trauma exposure.[48]

Because a salivary stress marker is widely accepted, low levels of cortisol in saliva were evaluated as a non-invasive measure to modulate stress responses. Apilux et al's development of a lateral current immunoassay device using a cortisol-BSA conjugate containing gold-labeled gold nanoparticles in a silver amplification system holds promise as an effective tool to detect cortisol associated with salivary stress.[52]

The assessment of salivary cortisol as a diagnostic biomarker in TBI is recommended. TBI may cause anxiety and stress in patients, and the positive correlation between stress and cortisol levels support its potential as a biomarker in TBI.[53]

Oxidative Stress

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced during natural physiological processes. ROS and RNS are highly reactive molecules that can damage key cell components. Under physiological conditions, the endogenous immune system is able to prevent the formation and aids in the removal of these harmful molecules protecting tissues against oxidative damage. Oxidative stress plays a crucial role in pathology. Typically, cells contain several antioxidants to counteract the damaging effects of oxidative chemicals, and a healthy biological balance between ROS and antioxidants must be maintained to prevent oxidative damage to cells and tissues.Any oxidative imbalance leads to the accumulation of oxidants causing oxidative damage to cells.[53,54]

In either case, changes in biomarkers can indicate the severity of the abnormality or the extent of the damage. The overall change in oxidative or antioxidant markers is a reliable indicator, and individual interpretation of each oxidative stress marker will be useful for a more accurate diagnosis and for understanding the overall trend.[53,54]

There is very little literature on the importance of antioxidants in TBI. Brain ascorbic acid decreased in TBI due to experimental explosion and was associated with decreased Glutathione and thiol protein and increased oxidative markers. Glutathione S-Transferases (GST) is an enzyme that exhibits glutathione peroxidase activity. Natural changes in GST expression affect neuronal degradation after experimental TBI and confirm the importance of lipid peroxidation as an important pathophysiological mechanism in TBI.[56,57]

Oxidative stress markers, including ROS and their mediators, have been evaluated in saliva samples. Salivary biomarkers have been shown to play a profound role in the diagnosis of many diseases or stressful conditions, including TBI.[58,59]

New insights into saliva cells as the main source of salivary biomarkers have prompted the development of new assay methods for standard measurement of biomarkers in fluid as well as cells. Digital Immunohistochemistry (IHC) and Western Blot (WB) for quantitative measurement of SRP 100 biomarker have been used for rapid detection.[60]

Other Biomarkers

Several other biomarkers including glial fibrillar acidic protein (GFAP), ubiquitin carboxy-terminal hydrolase L1 (UCH-L1), neuron-specific enolase (NSE), neurofilament light protein (NFL), beta-amyloid protein, lysozyme, immunoglobulins, lactoferrin, and others in saliva that may serve as diagnostic biomarkers in TBI patients need further studies.

Conclusions

More than half of all adults will be exposed to a traumatic event at some point in their lives, yet we do not yet have reliable biomarkers to help predict who experiences trauma-related symptoms in response to these events. In

this regard, it seems that several reliable, less expensive, and easily attainable biomarkers for TBI in saliva provide hope for early detection that may save lives.

This review of the literature has identified saliva biomarkers with the highest discriminative abilities as determined by operating characteristics in diagnostic situations in TBI. Saliva shows a strong promise for biomarkers of TBI based on new methods for saliva handling, analysis, and biomarker discovery.

The top performers in each biomarker may provide insight into pathogenic mechanisms of TBI that most influence the measured endpoint. Nonetheless, many challenges remain before these biomarkers can be incorporated into clinical practice. In particular, it remains unclear whether a large panel of biomarkers in addition to clinical assessment will be sufficient to stratify patients into categories of TBI before more specific biomarker assessments are applied.

With the refinement and validation of saliva biomarkers, we have the potential to use saliva biomarkers as a convenient and cost-effective surrogate for currently used imaging modalities for the evaluation of TBI in the clinical setting.

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Competing interests

The authors declared no potential conflict of interests with respect to the research, authorship, and/or publication of this article.

Abbreviations

Traumatic brain injury: TBI; Computed tomography scan: CT scan; Magnetic resonance imaging: MRI; National Institutes of Health: NIH; Noncoding RNAs: ncRNAs; Extracellular vesicles: EVs; MicroRNAs: miRNAs; Microtubule-associated protein tau: MAPT; Mixed martial arts: MMA; Glutathione S-Transferases: GST; Immunohistochemistry: IHC; Western Blot: WB; Reactive oxygen species: ROS; Reactive nitrogen species: RNS; Salivary alpha-amylase: sAA; Autonomic nervous system: ANS; Sympathetic nervous system: SNS.

Authors' contributions

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None.

Consent for publication

By submitting this document, the authors declare their consent for the final accepted version of the manuscript to be considered for publication.

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