Scientific Research Publishing

Open Journal of Modern Neurosurgery, 2019, 9, 356-368
http://www.scirp.org/journal/ojmn
ISSN Online: 2163-0585
ISSN Print: 2163-0569

Detection of the Severity of Brain Injury in Head Trauma Patients Using Biochemical Blood Markers and Its Correlation with Glasgow Coma Scale

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How to cite this paper: Ragaee, M.A., Ghandour, N.M. and Hanna, R.T. (2019) Detection of the Severity of Brain Injury in Head Trauma Patients Using Biochemical Blood Markers and Its Correlation with Glasgow Coma Scale. *Open Journal of Modern Neurosurgery*, **9**, 356-368.
https://doi.org/10.4236/ojmn.2019.93033

Received: June 30, 2019
Accepted: July 28, 2019
Published: July 31, 2019

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Abstract

Head trauma is one of common injury related mortality and morbidity. Blood biomarkers are valuable tools for the identification and characterization of initial injury and secondary pathological processes for traumatic brain injury (TBI). This study evaluated the performance of a recently developed visfatin and its correlation with other blood circulating biomarkers that reflect specific pathological mechanisms including neuro inflammatory, neuron injury and oxidative damage in moderate to severe TBI patients. Peripheral blood was taken from TBI patients (n = 78) at hospital admission, maximum 6 hours post-injury. Severity and neurological outcome were assessed using the Glasgow Coma Scale (GCS) and blood level of: visfatin, neuron specific enolase (NSE), malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione (GSH). Concentrations of visfatin (28 ± 1.68 μg/L, 25 ± 2.09 μg/L) was significantly higher ($p < 0.0001$) in sever and moderate groups of TBI patients respectively compared to control group (7.62 ± 0.87 μg/L), NSE concentrations also were significantly higher ($p < 0.0001$) in both groups of TBI patients (20.47 ± 3 ng/ml, 13.49 ± 2.66 ng/ml) compared to control group (4.3 ± 0.52 ng/ml), MDA was significantly elevated ($p < 0.001$) in sever TBI patients group (6.88 ± 0.58 μmol/L) compared to control group (5.12 ± 0.76 μmol/L), while SOD (245.12 ± 24.2 U/L, 276.097 ± 30.8 U/L) and GSH (112.07 ± 2.09 μmol/L, 119.26 ± 2.7 μmol/L) were highly significantly decreased ($p < 0.0001$) in TBI patients compared to control group (304.17 ± 27.17 U/L and 151.64 ± 9.9 μmol/L) respectively. Visfatin was positively correlated with NSE and MDA, while there was negative correlation with SOD and GSH. In conclusion blood level of visfatin in correlation with other blood

biomarkers can be used for prediction of severity of TBI cases.

Keywords

Brain Injury, Visfatin, Biomarkers, Head Trauma

1. Introduction

Traumatic brain injury (TBI) is a critical public health problem throughout the world. It will become the third cause of death and disability in the general population by the year 2030. Early determination of injury severity is important in order to improve care, balance benefits and risks of early treatment options. Existing predictors include age, Glasgow Coma Scale (GCS), pupil response to light and size, and grading of extent/type of TBI damage on imaging [1] [2] [3].

In recent years, researches have been devoted to finding biomarkers that can improve the predictive capacity of demographic, clinical and imaging factors. Kövesdi *et al.* ascertained that to be specific and sensitive, biomarkers should be proportional to the mechanical impact and the extent of the injury, as well as, appear rapidly in the blood [4] [5].

The most established approach to develop biomarkers for brain trauma is to identify proteins abundant in brain cells e.g.: neuron-specific enolase (NSE) and Ubiquitin C-terminal hydrolase-L1 (UCH-L1) in neurons; S100B and glial fibrillary acidic protein (GFAP) in astroglia. The second approach is to study inflammatory cytokines, oxidized lipids and metabolites [6].

NSE is a glycolytic protein. It can be used as an indicator of functional levels and mortality rates after brain injury. This protein is passively released into the extracellular space only under pathological conditions during cell destruction and its concentration rises to its peak 6 hours after the injury [7] [8] [9].

Visfatin is a newly identified pro-inflammatory adipokine which was found to be produced and secreted in visceral fat. Many researchers reported that it has a close relationship with inflammation and tissue damage repair. Circulating visfatin concentrations have been reported to be elevated in patients with type 2 diabetes mellitus, obesity, ischemic stroke and rheumatoid arthritis [10] [11].

The brain is highly sensitive to oxidative stress. Under physiological conditions, the defense system is able to prevent the formation or scavenge of these harmful molecules, protecting tissues from oxidative damage. In brain injury, there is a considerable increase in the production of free radicals [12].

The main objective of this study is to evaluate the changes of plasma visfatin level in patients with TBI and the relation of these changes with GCS of the patients (as a clinical indicator of the severity of the head trauma), and its correlation to changes of other blood biomarkers e.g. neuron specific enolase (NSE), Malondialdehyde (MDA), super oxide dismutase (SOD) and glutathione (GSH). Also, this study aimed to estimate the possible value of these markers for the prediction of TBI severity.

2. Patients and Methods

Patients, Design and Procedures

This is an observational descriptive study. The study group included 78 brain trauma patients admitted to the Department of Neurosurgery, Assiut University Hospital. Upon admission at trauma emergency room, patients were evaluated General and Neurological and First aid management done, then we start to select patients according to inclusion and exclusion criteria for the purpose of this research.

2.1. Inclusion Criteria

All patients presented to trauma unit in Assiut university hospital during the period from June 2018 to February 2019,

- With only head trauma without other associated body trauma.
- Age more than 18 years of age.
- Trauma within the last 6 hours.

2.2. Exclusion Criteria

The excluded patients were described by Papa *et al.*:

- Patients less than 18 years of age.
- Admission time > 6 h.
- Patients with associated other body trauma.
- Existing previous head trauma, neurological disease including ischemic or hemorrhagic stroke—Presence of prior systemic diseases as liver cirrhosis, diabetes mellitus, malignancy, chronic heart or lung disease.
- Any condition that cause hemolysis, hypertension and obesity as well as use of anticoagulant medication [8].

We used the GCS as the main indicator of the severity of the head trauma to clinically evaluate each patient at time of admission and after resuscitation and first aid management.

The patients were divided according to GCScriteria. GCS (13-15) considered mild; GCS (9-12) moderate while GCS (3-8) considered severe injury. Head CT scan and basic blood investigations were done for all patients [13].

Patients medical history was taken from patients first degree relatives. Demographic data (age, sex) was obtained.

Blood samples were taken from all patients just after admission for basic blood investigations including complete blood picture, prothrombin time, concentration and INR, blood gases, serum electrolytes, renal function and liver function to exclude any other renal, liver or blood disease.

To highlight the results, 20 healthy people were enrolled as control group. All control individuals investigated properly to exclude the presence of any chronic disease like renal, liver, cardiac, blood, hypertension, morbid obesity, CNS disease and previous head trauma [13].

Blood sample collection and analysis:

Venous blood (10 ml) was drawn, from patients on admission and from control group, into EDTA glass tubes. 8 ml were centrifuged at 1500 round per minute (rpm) for 20 min under room temperature to collect plasma. Plasma was stored at −70 C until analysis of different biochemical markers.

1) Measurement of visfatin and Neuron specific Enolase (NSE):

Enzyme linked immunosorbent assay technique (ELISA) was performed to measure plasma concentrations of Visfatin and NSE, using commercial kit (Cat No. 30092, RayBiotech, 3607, Parkway Lane Suite 200, Norcross) for Visfatin and commercial kit (Cat no. 153308, MyBioSource, P.O. Box San Diego, California, United States) for NSE following the instructions supplied with the kits. Samples were measured as duplicate for single experiment.

2) Measurement of oxidative markers:

Analysis of Lipid peroxides malondialdehyde derivative (MDA), as an index for oxidative stress, superoxide dismutase (SOD) and glutathione (GSH), as antioxidants, were done by spectrophotometry.

Malondialdehyde derivative was determined by chemical method according to Ohkawa *et al.* which based on the oxidation of zylenol orange into purple colored chromogen that is proportional with the peroxide content in the presence of ferrous sulfate as a catalyzer. It was measured at 532 nm in spectrophotometer [14].

Superoxide dismutase activity was determined according to its ability to inhibit the auto-oxidation of epinephrine at alkaline media as described by Misra and Fridovich (1972). Spectrum was measured at 480 nm [15].

Glutathione was assayed according to method of Jollow *et al.* (1974). which relies on mixing 1 mL of sample with 1.0 mL of sulfosalicylic acid (4%). The samples were incubated at 4˚C for at least 1 h, and then centrifuged at 1200 rpm for 15 min at 4˚C. The reaction mixture contained 0.4 mL of filtered sample, 2.2 mL phosphate buffer (0.1M, pH 7.4), and 0.4 mL dithionitrobenzoic acid (DTNB) in a total volume of 3 mL. The yellow color developed was read immediately at 412 nm by spectrophotometer [16].

2.3. Statistical Analysis

Statistical analysis was performed with SPSS 20.0 (SPSS Inc. Chicago, USA).

The categorical variables are presented as percentages, and the continuous variables are presented as mean ± standard deviation. Comparisons were made using Chi-square test or Fisher exact test for categorical data and unpaired Student t-test and Mann-Whitney for continuous variables. When $p < 0.05$, the difference was statistically significant. The correlation, of visfatin with other markers, was assessed by Spearman's correlation coefficient.

2.4. Ethical Considerations

The study was reviewed and approved by Research and Ethical Committee of Faculty of Medicine, Assiut University. Written informed consent was taken from control group and relatives of TBI who participated in the study. Confi-

dentiality of the data was guaranteed.

3. Results

3.1. Patient Characteristics

Seventy-eight patients, were admitted to Neurosurgery department with an isolated head trauma, were included in the study. Twenty healthy people were included as control group. Patients were 50 males and 28 females with mean age of (52.38 ± 1.65) years. The control group was 13 males and 7 females with mean age of (53.86 ± 2.7). There were no significant differences in both sex and age among patients and control subjects (both $p > 0.05$). These characteristics were listed in Table 1. Regarding GCS criteria, they were 52 moderate TBI patients and 26 sever TBI patients. The causes of TBI and CT scan results of patients were shown in Table 2. Motor car accident (MCA) represented the major cause of trauma in 20 cases (25.6%), followed by motor bike accident (MBA) in 16 cases (20.5%), while Fall on ground (FOG) and Fall on stairs (FOS) were the cause in 4 injuries (5.1%) for each. Regarding CT scan results, sixteen patients (20.5%) showed brain edema, and eight cases (10.3%) showed contusion. Extradural hemorrhage represented the highest type of intracranial hemorrhage among included patients, in 20 cases. Depressed skull fracture was found in 6 cases (7.7%).

3.2. Biochemical Markers

Visfatin concentration was significantly elevated ($p < 0.001$) in severe TBI patients as compared to moderate TBI patients (28 ± 1.68 μg/L, 25 ± 2.09 μg/L) respectively. Its concentration was highly significantly elevated ($p < 0.0001$) in both TBI groups as compared to control (7.62 ± 0.87 μg/L). Also; NSE concentration was significantly higher ($p < 0.0001$) in severe TBI patients as compared to moderate TBI patients (20.47 ± 3 ng/ml, 13.49 ± 2.66 ng/ml) respectively. Its concentration was highly significantly elevated ($p < 0.0001$) in both TBI groups as compared to control (4.3 ± 0.52 ng/ml) as shown in Table 3.

MDA was significantly elevated ($p < 0.001$) in severe TBI patients as compared to moderate TBI patients (6.88 ± 0.58 μmol/L, 5.33 ± 0.08 μmol/L) respectively. It was significantly elevated ($p < 0.001$) in severe TBI patients when compared to control (5.12 ± 0.76 μmol/L), while, this elevation was non-significant in moderate TBI patients compared to control. Regarding SOD, its concentration was significantly decreased ($p < 0.01$) in severe TBI patients as compared to moderate TBI patients (245.12 ± 24.2 U/L, 276.097 ± 30.8 U/L). Glutathione was highly significant decreased ($p < 0.0001$) in severe TBI patients as compared to moderate TBI patients (112.07 ± 2.09 μmol/L, 119.26 ± 2.7 μmol/L). Both, SOD and GSH, were highly significantly decreased ($p < 0.0001$) as compared to control (304.17 ± 27.17 U/L and 151.64 ± 9.9 μmol/L) respectively, as shown in Table 4.

The correlation of plasma visfatin concentration in TBI patients and other bi-

ochemical parameters was illustrated in Table 5. Visfatin was positively correlated with NSE ($r = 0.699$, $p < 0.01$) and MDA ($r = 0.338$, $p < 0.05$). While there was negative correlation with SOD ($r = -0.156$, non-significant) and GSH ($r = -0.588$, $p < 0.01$).

4. Discussion

Head trauma is one of common injury related mortality and morbidity. Traumatic brain injury patients represent the greatest challenge for accurate diagnosis and outcome prediction especially in the acute setting "within 24 h after injury" [17].

Table 1. Characteristics of the studied groups.

	Patients (n = 78) Mean ± SD	**Control (n = 20)** Mean ± SD	**p value**
Age	52.38 ± 1.65	53.86 ± 2.7	ns (p = 0.647)
Sex	50 males (64.1%) 28 females (35.9%)	13 males (65%) 7 females (35%)	ns (p = 0.752)

SD: Standard deviation, n: number, ns: non-significant.

Table 2. Causes of trauma and results of head CT scan in TBI patients (n = 78).

Causes of trauma		n	%
RTA		10	12.8
MCA		20	25.6
MBA		16	20.5
FOG		4	5.1
FFH		8	10.3
FOS		4	5.1
AFO		8	10.3
HO		8	10.3
	Ct scan results		
Brain	**Edema**	16	20.5
	Contusion	8	10.3
Intracranial hemorrhage	**SAH**	12	15.4
	EDH	20	25.6
	ASDH	6	7.7
Fracture	**Fissure**	5	6.4
	Depressed	6	7.7
	Base	5	6.4

CT: Computed Tomography; TBI: Traumatic brain injury; n: Number; RTA (Road traffic accident); MCA (Motor car accident); MBA (Motor bike accident); FOG (Fall on ground); FFH (Fall from height); FOS (Fall on stairs); AFO (Assault from other); HO (heavy object); SAH (subarachnoid hemorrhage); EDH (extradural hemorrhage); ASDH (acute subdural hemorrhage).

Table 3. Plasma levels of visfatin and NSE in studied groups (moderate injured patients, severe injured patients and control).

Biochemical Markers Cases		Visfatin (μg/L) Mean ± SD	Enolase (NSE) (ng/ml) Mean ± SD
Patients (n = 78)	Moderate (n = 52)	25 ± 2.09 $^{\#\#\#}$($p < 0.0001$)	13.49 ± 2.66 $^{\#\#\#}$($p < 0.0001$)
	Severe (n = 26)	28 ± 1.68 **($p < 0.001$) $^{\#\#\#}$($p < 0.0001$)	20.47 ± 3 ***($p < 0.0001$) $^{\#\#\#}$($p < 0.0001$)
Control (n = 20)		7.62 ± 0.87	4.3 ± 0.52

SD: Standard deviation, n: number, NSE: Neuron specific enolase. *($p < 0.01$) **($p < 0.001$) ***($p < 0.0001$) significant difference as compared to moderately injured patients. $^{\#}$($p < 0.01$) $^{\#\#}$($p < 0.001$) $^{\#\#\#}$($p < 0.0001$) significant difference as compared to control.

Table 4 Plasma levels of MDA, SOD and GSH in studied groups (moderate injured patients, severe injured patients and control).

Biochemical Markers Cases		MDA(μmol/L) Mean ± SD	SOD(U/L) Mean ± SD	GSH(μmol/L) Mean ± SD
Patients (n = 78)	Moderate (n = 52)	5.33 ± 0.08$^{ns\#}$ ($p = 0.631$)	276.097 ± 30.8$^{\#\#}$ ($p < 0.001$)	119.26 ± 2.7$^{\#\#\#}$ ($p < 0.0001$)
	Severe (n = 26)	6.88 ± 0.58 **($p < 0.001$) $^{\#\#}$($p < 0.001$)	245.12 ± 24.2 *($p < 0.01$) $^{\#\#\#}$($p < 0.0001$)	112.07 ± 2.09 ***($p < 0.0001$) $^{\#\#\#}$($p < 0.0001$)
Control (n = 20)		5.12 ± 0.76	304.17 ± 27.17	151.64 ± 9.9

MDA: Malondialdehyde, SOD: Superoxide dismutase, GSH: Glutathione, SD: Standard deviation, n: number, ns non-significant. *($p < 0.01$) **($p < 0.001$) ***($p < 0.0001$) significant difference as compared to moderately injured patients. $^{\#}$($p < 0.01$) $^{\#\#}$($p < 0.001$) $^{\#\#\#}$($p < 0.0001$) significant difference as compared to control.

Table 5. Correlation between Plasma visfatin concentration and other biochemical markers in traumatic brain injured patients:

Biochemical Markers	Enolase (NSE)	Malondialdehyde (MDA)	Superoxide dismutase (SOD	Glutathione (GSH)
Visfatin	0.699** ($p < 0.01$)	0.338* ($p < 0.05$)	−0.156^{ns} ($p = 0.266$)	−0.588** ($p < 0.01$)
Enolase (NSE)		0.484** ($p < 0.01$)	−0.531** ($p < 0.01$)	−0.717** ($p < 0.01$)
Malondialdehyde (MDA)			−0.221^{ns} ($p = 0.112$)	−0.530** ($p < 0.01$)
Superoxide dismutase (SOD)				0.146^{ns} ($p = 0.295$)

NSE: Enolase, MDA: Malondialdehyde, SOD: Superoxide dismutase, GSH: Glutathione, Spearman Correlation/Sig. (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed). *. Correlation is significant at the 0.05 level (2-tailed).

Prognosis can be assessed by many predictors such as age, neurological examination of some clinical variables e.g. Glasgow Coma Scale (GCS), presence of

Neurological deficit, pupils size and reaction to light. In addition, neuroimaging tools such as MRI and CT scanning can be used [18].

In brain trauma, blood biomarkers are preferable than CSF for accurate and rapid assessments, as they are cost effective; and require minimally invasive sample collection, compared to CSF collected by invasive method as lumbar puncture (LP) or ventriculostomy [19].

Circulating serological biochemical markers may be associated with secondary injury progression and poor prognosis. A single factor has insufficient predictive value to distinguish patients who will do poorly from those who will be good. Thus, for accuracy, a multi-marker approach to characterize traumatic brain injured patients outcome has been advocated and may increase diagnostic and prognostic accuracy and may prove multi-systemic character of secondary injury pathology [13] [20].

This research aimed to diagnose traumatic brain injury by assessing five blood biomarkers, each associated with a specific traumatic brain related injury process: Visfatin as pro-inflammatory adipokine, neuron specific enolase (NSE) relating to neuronal injury; lipid peroxides malondialdehyde derivative (MDA) for oxidative stress; superoxide dismutase (SOD) and glutathione (GSH), as antioxidants. Also, to correlate the change in plasma visfatin to other biomarkers, as well as, to clinical severity after traumatic brain injury, for which, no published information exists to date about this.

Seventy-eight patients were included in the study. Males were more than females (as most TBI cases were due to outdoor activities). Regarding GCS criteria, they were 52 moderate TBI patients and 26 severely injured. No mild cases were evaluated, as Assiut University Hospital (AUH) is a big referral center for all Upper Egypt governorates. The emergency units in AUH are tertiary level units receiving all types of trauma, either direct or referred from other hospitals. Therefore, they admit patients to undergo operative treatment or admitted to the intensive care in trauma unit [21].

Visfatin has a vital role in the pathogenesis of vascular inflammation and it was closely related with inflammation and repair after tissue damage. Thrombin and fibrin degradation products can induce a series of inflammatory reactions after traumatic brain injury, so the generation and the concentration of most inflammatory factors increase [22] [23].

In the presented study, Visfatin concentration was significantly elevated in severe TBI patients as compared to moderate TBI patients. Its concentration was highly significantly elevated in both TBI groups as compared to control.

This was consistence with Chen *et al.*, who reported for the first time significantly higher visfatin levels in patients with severe TBI. In addition, Weng *et al.* demonstrated highest plasma visfatin and C-reactive protein concentrations among head injured patients [24] [25].

NSE is a marker present in the neuron cell body. Ogata and Tsuganezawa ascertained that NSE is a surrogate marker of neuronal damage. They found ele-

vated NSE with injured axons in the corpus callosum of patients sustaining fatal diffuse axonal injury, while, NSE is nearly undetectable in patients with non-injured axons or control subjects [26] [27].

The results of this study showed that NSE concentration was also, significantly higher in severe TBI patients as compared to moderate TBI patients. Its concentration was highly significantly elevated in both TBI groups as compared to control.

In agreement with the presented study, Lee *et al.* reported that NSE could be used as an indicator of functional levels and mortality rates after head injury. Vos *et al.* found elevated NSE levels, correlated with the injury severity score and CT findings and were significantly higher in patients with poor outcome post injury. McKeating *et al.* found that NSE in serum peaks within 6 h after injury and decreases during the subsequent hours. Multiple studies have shown that serum NSE levels spike after moderate to severe head injury [28]-[36].

In addition, Di Battista *et al.*, found unfavorable neurological outcome was associated with elevations in six of the seven markers including neuron specific enolase (NSE), and lipid peroxidation [13].

In controversy with that, Shahim *et al.* reported no difference in serum NSE in post-concussion values among 35 concussed ice hockey players. This may be explained as in concussion there is no actual damage or injury to brain cells, hence there is no elevation of its level [37].

One of the major factors that can cause tissue damage initiated by head trauma is lipid peroxidation. There are intracellular lactate production and hyperglycaemia, during the acute ischemic phase of brain injury, which leads to subsequent development of reactive oxygen species [38] [39] [40].

In this study, MDA, as an indicator of lipid peroxidation, was significantly elevated in severe TBI patients as compared to moderate TBI patients. While, this elevation was non-significant in moderate TBI patients, it was significantly elevated in severe TBI patients when compared to control. Regarding superoxide dismutase (SOD) and glutathione (GSH) as antioxidants, SOD concentration was significantly decreased in severe TBI patients as compared to moderate TBI patients. Glutathione, was highly significant decreased in severe TBI patients as compared to moderate TBI patients. Both, SOD and GSH, were highly significantly decreased when compared to control.

Similarly, Kasprzak *et al.* demonstrated that increased erythrocyte TBARS concentrations were correlated with the severity of injury in the patients with brain contusion. This elevation in lipid peroxidation was associated with unfavorable neurological outcome [13] [41].

Rodriguez *et al.* stated that when the tissues are exposed to oxidative stress, they increase the expression of antioxidant enzymes as a compensatory mechanism against free radical-mediated damage. And they ascertained that the increased activity of the antioxidant enzymes may be inadequate to counteract the potential damage in many conditions of oxidative stress. So, the decreased levels

of antioxidants in the presented study lead to highly elevated oxidative stress conditions [42].

This consistent with Ozdemir *et al.* who found that whereas TBI significantly increased thiobarbituric acid reactive substances (TBARS) levels, there was no compensatory increase in SOD and glutathione peroxidase (GPx) 24 hours after TBI in 7-day-old rats. In addition, the depleted level of glutathione in the tissues may lead to progressing oxidative stress and more complications [2] [12] [43].

Studies have shown that clinical practice guidelines have an impact on the outcomes of trauma cases. Hence, it is of great importance to incorporate new modalities of diagnosis and prognosis in evaluation of medical care [44] [45].

We are currently working on evaluation of the role of visfatin gene mutation in patients with TBI, and its correlation to changes of other blood biomarkers e.g. neuron specific enolase (NSE), Malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione (GSH).

Also, we are working in evaluation of patient's outcome and its relation to previously detected blood biomarkers.

5. Conclusion

Traumatic brain injury can release a number of injury related molecules which are easily assessed in the peripheral blood. These biomarkers could facilitate diagnosis and may hold promise in the clinical management of patients. This research studied the predictive performance of some circulating biomarkers which reflect specific pathological mechanisms including neuro inflammation, neuron injury and oxidative damage in moderate to severe traumatic brain injury patients.

Recommendations

- Evaluation of the prognostic utility of the change in blood levels of these biomarkers in mild injured patients, for longer-term outcome and in large enough sample size.
- Evaluation of patient's outcome and its relation to previously detected blood biomarkers.
- Evaluation of the relation of blood levels of these biomarkers and the type of brain injury detected by imaging techniques (CAT Scan and MRI).

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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