Glial Fibrillary Acidic Protein (GFAP) and Cleaved Tau Protein (CTP) Biomarkers as a Forensic Tool for Detection and Assessement of Traumatic Brain Injury

[1]Mohamed Kamel Mohamed[1], Mervat Hamdy Abd Elsalam[2], Eman Abd Elfattah mohammed Elzohairy[2], Dina Sabry Abd Elfattah[3], Ehab Abd Elhalim Abd Elsalam[4]

[1]Assistant Lecturer of Forensic Medicine and Clinical Toxicology, Faculty of Medicine Cairo University, [2]Professor of Forensic Medicine and Clinical Toxicology, Faculty of Medicine Cairo University, [3]Professor of Biochemistry, Biochemistry Department Faculty of Medicine Cairo University, [4]Lecturer of Neurosurgery, Neurosurgery Department Faculty of Medicine Cairo University, Cairo University, Kasr-Alainy Street, Cairo 11562, Egypt

Abstract

Traumatic brain injury (TBI) is defined as a traumatically-induced structural brain injury or physiological disruption of brain function caused by an external force. Traumatic brain injury is a neuropsychiatric disorder that breaks down the remaining barriers between neurology and psychiatry. Several biomarkers have been developed to directly determine the pathology of the nerve cells in the central nervous system (CNS) when it is injured. This review recent research on brain injury biomarkers could be used for rapid and accurate diagnostics of TBI in easily accessible fluid.

Objectives: The purpose of this study was to assess utility of GFAP-BDP for the diagnosis of intracranial injury in patients with a positive clinical screen for head injury across the spectrum of TBI typically presenting to ED in Kasr- Alainy hospital.

Subjects & Methods: This prospective cohort study was based on the data collected from 90 cases presented to Kasr Al-Aini Hospitals Emergency Department, Cairo University, with history of traumatic brain injury through the period from April 2017 to Mars 2019. According to age, they were classified into 3 age groups; age group A (18-35 years), age group B (36-50 years) and age group C (> 50 years). Data were analyzed with respect to socio-demographic data, type of head injury, clinical presentations, radiological investigation, and management of TBI in relation to serum specific biomarkers level (GFAP, C-tau).

Results: The most common age group was age group B (18-35 years) (70%). Males were more common than females (71.1% and 28.9% respectively). The most common cause of trauma was fall from height (33.3%). Serum GFAP level and C-tau levels in studied groups show high significant correlation between them (p value <0.001).

Conclusion and Recommendations: The combination of the two biomarkers or more may be more useful than either biomarker in isolation for predicting intracranial lesions on CT scanning therefor decreases unwanted CT head scanning and radiological bad effect especially in young ages.

Keywords: *Traumatic brain injury, Serum, GFAP, C-tau and CT scanning*

Corresponding author:
Mohamed Kamel Mohamed
Assistant lecturer of forensic medicine and clinical toxicology faculty of medicine Cairo University
Drmkamel315@gmail.com, 01007556847
Giza, el Eshreen Street, Egypt

Introduction

TBI is a common problem, called a silent epidemic because of a general unawareness of the condition. TBI is difficult to diagnose with imaging techniques, and there is no definite laboratory test to support the diagnosis. An undiagnosed case of TBI can result in premature

return to play with severe consequences or in a chronic neurodegenerative condition later in life.[1]

Traumatic brain injury (TBI) is defined as a traumatically-induced structural brain injury or physiological disruption of brain function caused by an external force.[2]

An ideal laboratory test, detecting a brain injury–specific biomarker in one of the body fluids, would confirm or rule out the TBI, predict the outcome, and indicate when recovery is complete. This review recent research on brain injury biomarkers could be used for rapid and accurate diagnostics of TBI in easily accessible fluid.[3]

Head CT scan is the diagnostic modality of choice to evaluate patients for traumatic intracranial injuries. Although effective for detecting traumatic injuries that require observation or neurosurgical evacuation due to the widespread use of head CT scanning has been questioned due to potential adverse effects of radiation exposure, unnecessary emergency department resource use, and cost.[4]

Several biomarkers have been developed to directly determine the pathology of the nerve cells in the central nervous system (CNS) when it is injured.[5]The blood–brain barrier (BBB), which normally is almost impermeable, can lose its integrity upon brain injury and allow the permeation of molecules into the blood.[6]

A number of patients who suffer from mild head injury later on develop significant disabilities. Biomarkers help identify and quantify the extent of injury and help predict the possible functional outcome of the patients.[7]

Materials and Method

Study population:

Data collected from 90 cases presented to Kasr Al-Ainy emergency department -according to specific criteria-during the period of the study. Egyptian origin and residence, more than 18 years, both sexes with history of traumatic brain injury were included in the study. While patients who had any pre-morbid non traumatic neurologic conditions or history of previous brain trauma were excluded from the study.

· Selected patients presenting within 24 hours of injury were subjected to draw (5cm) blood samples and dated to compare with time of injury. Serum samples were extracted and preserved at -20°C till chemical analysis.

· Selected patients were classified to groups according to age:

1. Group (A) 18-35 years.
2. Group (B) 36-50 years.
3. Group (C) >50 years

· Another classification according to Glasco Coma Scale into:

1. Mild TBI (GCS 13-15).
2. Moderate TBI (GCS 9-12). The study cannot depend on CT head as it is not informative.

· All patients had at least one CT head scan at admission time.

· Time of traumatic brain injury time obtained from patients themselves, patient relatives, ambulance crew, and others (referral letter, police report).

· The study will measure the level of GFAP and Cleaved Tau Protein (CTP) by ELISA a proprietary mouse monoclonal antibody for solid phase immobilization, and a proprietary polyclonal rabbit antibody for detection.

Data Collection

The current study sample collection process is designed to be taken in first 24 hours after trauma causing brain trauma. Negative CT head patients presented to emergency department with history of TBI must be observed at least 24 hours and another CT head may be further more indicated. Study of brain specific biomarkers will help doctors to decrease unnecessary CT head, decrease period of hospital stay and give them protection form medical liability if deterioration or death after uncalculated discharge has been occurred.

It also, divides 24 hours after trauma (total time of sample collection) to three subgroups to give more prediction accuracy to diagnose and evaluate TBI using brain specific biochemical markers level in relation to

delay time from trauma to sample collection.

The data will be formulated according to the data revealed from:

§ Patients primary survey (clinical examination and radiological CT scan)

§ Laboratory investigations (serum GFAP and C-tau levels). All cases presented with history of TBI within 24h of the injury.

I. Demographic data:

· Age: classified according to group A, B, and C.

· Sex: Males & Females.

II. Clinical picture:

· **GCS score:** 13-15 points, 9-12 points.

· **Pupil light reflex:** Normal, Sluggish.

· **Vomiting:** Yes, No.

· **Severity of injury:** Mild, Moderate.

III. Radiological investigations:

· **CT head scan findings:** Positive, Negative**.**

· **MRI brain:** Not indicated, Indicated.

IV. lab investigations:

· **GFAP level:** <200, 200-499, 500-999, 1000-1999, >2000.

· **C-tau level:** <100, 100-149, 150-199, 200-300, >300.

V. Management: Conservative, urgical.

Ethical considerations

The study approval was taken by the ethical review committee of medical research, Faculty of Medicine, Cairo University, Egypt and informed consent forms were obtained from all participants. The study information including the purpose and details were explained to participants of both groups.

Results

Table 1 showed that 11.1% (10 patients) of cases had GFAP level below 200 and 45.6 % (41 patients) of cases had level between 200-499, 27.8 % (25 patients) of cases had level between 500-999, 14.4 % (13 patients) of cases had level between 1000-2000 and finally 1.1 % of cases had level more than 2000. The results showed that 5.6% (5 patients) of cases had C-tau level below 100 and 45.6 % (41 patients) of cases had level between 100-149 then 18.9 % (17 patients) of cases had level between 150-199 then 20.0 % (18 patients) of cases had level between 200-300 finally 10.0 % (9 patients) of cases had level more than 300

Table 1: Serum GFAP and C-tau levels among studied cases

GFAP level(pg./ml)	No.	%
<200	10	11.1
200-499	41	45.6
500-999	25	27.8
1000-1999	13	14.4
>2000	1	1.1
Mean ± SD	603.9±443.9	
Median (Range)	477.0(93-2995)	

Cont... **Table 1: Serum GFAP and C-tau levels among studied cases**

C-tau LEVEL(pg./ml)	No.	%
<100	5	5.6
100-149	41	45.6
150-199	17	18.9
200-300	18	20
>300	9	10
Mean ± SD	184.7±92.6	
Median (Range)	174.0(78.0-597.0)	

Table 2 Comparing pupil light reflex, GCS and management hospital stay, with GFAP level among studied cases, there was statistical difference between mean GFAP biomarker level which was highly significant (p value 0.001 0.001, 0.005, 0.023).

Table 2: GFAP level in relation to pupil, vomiting, GCS and management (mean ± SD) using ONE way ANOVA

GFAP							
		Mean	**±SD**	**Median**	**Minimum**	**Maximum**	**p value**
Pupil	Normal	550	447	424	93	2995	0.001*
	Sluggish	769	401	626	200	1732	
Vomiting	No	541	324	477	93	1425	0.322
	Yes	707	582	477	132	2995	
G C S	13-15	547	439	427	93	2995	0.001*
	9-12	815	405	639	348	1732	
Management	Conservative	574	494	418	93	2995	0.005*
	Surgical	664	322	590	219	1732	

****P-value < 0.05 is statistically significant P-value < 0.001 is statistically highly significant, and P-value ≥ 0.05 is statistically insignificant***

Table 3 Comparing management with C-tau level among studied cases, there was statistical difference between mean C-tau which was highly significant (p value <0.001)

C-tau							
		Mean	**±SD**	**Median**	**Minimum**	**Maximum**	**p value**
Pupil	Normal	182.7	95.6	142..0	78.0	597.0	0.464
	Sluggish	190.9	84.4	154.5	105.0	374.0	
Vomiting	Yes	176.6	80.8	144.5	78.0	459.0	0.398
	No	198.1	109.3	154.5	84.0	597.0	
G C S	13-15	179.6	94.1	141.0	78.0	597.0	0.134
	9-12	203.7	86.4	163.0	115.0	374.0	
Management	Conservative	180.0	96.0	144.5	78.0	597.0	<0.001*
	Surgical	194.0	85.8	153.0	108.0	374.0	

Table 3: C-tau level in relation to pupil, vomiting, GCS and management (mean ± SD) using ONE way ANOVA

P-value < 0.05 is statistically significant P-value < 0.001 is statistically highly significant, and P-value ≥ 0.05 is statistically insignificant

Table 4 Comparing CT head and severity of injury with GFAP level among studied cases, there was statistical difference between mean GFAP level which was significant p value (<0.001, 0.001).

Table 4: GFAP level in relation to CT head, severity of injury, MRI brain and head fracture (mean ± SD) using ONE way ANOVA

GFAP							
		Mean	**±SD**	**Median**	**Minimum**	**Maximum**	**p value**
C T head	Positive	811	483	612	219	2995	<0.001*
	Negative	333	144	342	93	733	
Severity	Mild	547	439	427	93	2995	0.001*
	Moderate	815	405	639	348	1732	
M R I	Not indicated	611	455	475	93	2995	0.795
	Indicated	548	359	483	200	1497	

*P-value < 0.05 is statistically significant P-value < 0.001 is statistically highly significant, and P-value ≥ 0.05 is statistically insignificant

Table 5 Comparing CT head with C-tau level among studied cases, there was statistical difference between mean C-tau level which was significant (p value 0.026).

Table 5: C-tau level in relation to CT head, severity of injury, MRI brain and head fracture (mean ± SD) using ONE way ANOVA

C-tau							
		Mean	**±SD**	**Median**	**Minimum**	**Maximum**	**p value**
C T head	Positive	205.1	106.3	163.0	102.0	597.0	0.026*
	Negative	158.0	62.8	139.0	78.0	358.0	
Severity	Mild	179.6	94.1	141.0	78.0	597.0	0.134
	Moderate	203.7	86.4	163.0	115.0	374.0	
M R I	Not indicated	182.3	94.2	144.5	78.0	597.0	0.261
	Indicated	204.4	80.3	197.5	115.0	358.0	

P-value < 0.05 is statistically significant P-value < 0.001 is statistically highly significant, and P-value ≥ 0.05 is statistically insignificant

Table 6 showed Pearson correlation between GFAP level and C-tau level in studied groups there was high significant correlation between them (p value <0.001).

Table 6: Pearson correlation between GFAP and C-tau TBI biomarkers level in studied cases.

	Pearson Correlation	**p -value**
GFAB level and C.tau level (pg./ml)	0.402	<0.001*

Discussion

Regarding GFAP level in studied cases, the study showed that 11.1% (10 patients) of cases had GFAP level below 200 and 45.6 % (41 patients) of cases had level between 200-499 then 27.8 % (25 patients) of cases had level between 500-999 then 14.4 % (13 patients) of cases had level between 1000-1999 finally 1.1 % (1 patients) of cases had level more than 2000. Most of studied cases had a level 200-499.

This is in agreement with [8] who reported that GFAP levels were significantly higher in those with evidence of traumatic pathoanatomic CT features when compared subjects with a negative head CT scan. (CT negative, mean GFAP 0.26±0.41 ng/mL; CT positive, mean GFAP 2.88±3.74 ng/mL; p<0.01)

Regarding C-tau level in studied cases, the study showed that 5.6% (5 patients) of cases had C-tau level below 100 and 45.6 % (41 patients) of cases had level between 100-149 then 18.9 % (17 patients) of cases had level between 150-199 then 20.0 % (18 patients) of cases had level between 200-300 finally 10.0 % (9 patients) of cases had level more than 3000. Most of studied cases had a level 100-149.

[9] reported that the combination of the two biomarkers or more may be more useful than either biomarker in isolation for predicting intracranial lesions on CT scanning.

Regarding pupil light reflex, GCS, management, duration of hospital stay with GFAP level among studied cases, there was statistical difference between mean GFAP biomarker level which was highly significant (p value 0.001 0.001, 0.005, 0.023).

The current study is in agreement with [10] who studied multivariable prognostic analysis in traumatic brain injury as expected, both the GCS motor score and pupil response are powerful independent predictors of outcome. GCS also can predict the line of treatment and estimated period of hospital stay.

[11] who studied Glial fibrillary acidic protein as a biomarker in severe traumatic brain injury patients the study showed similar results that by pupil light reflex examination of TBI presented cases, reflex was normal in 80% of patients. The prediction value of pupil light reflex test was higher than other clinical variables such as age, occupation, and vomiting in diagnosis and evaluation of traumatic brain injuries.

The study concluded that, patients with lower GCS had significantly higher level of serum C-tau protein and were associated with poor outcome. Correlating the serum C-tau levels and the GCS points at which it is significant may guide us further in qualitatively analysing the significant serum tau levels and throw some light on its role in projecting TBI prognosis.

[9] who studied acute biomarkers of traumatic brain injury, the mean C-tau biomarker levels differ between moderate to severe TBI (GCS 3–12) and mild TBI (GCS 13–15) and also differ between complicated mild TBI (GCS 13–15 with abnormal cranial CT) and uncomplicated mild TBI.

Regarding CT head and severity of injury with GFAP level among studied cases, there was statistical difference between mean GFAP level which was significant p value (<0.001, 0.001) Table (51).

Our results are in accordance with [8] who proved that, the ability of the GFAP level to discriminate between patients with mild and moderate-to-severe injuries, as measured by the AUC, was 0.87 (95% CI, 0.81–0.93). The discriminatory ability of GFAP in assessing mild-moderate versus severe injury was 0.84 (95% CI, 0.77–0.91).

This is results are in accordance to [12] who suggested that, in patients with traumatic intracranial lesions on CT head scan, GFAP levels were significantly elevated (median, 0.588ng/mL), compared with those without lesions (median 0.033ng/mL).

In the present study, mean serum C-tau level in CT positive group was significantly higher compared to CT negative group. These results signify that this increase in serum C-tau level may be used to discriminate between patients with intracranial lesions and those without intracranial lesions, irrespective of the severity of injury.

[13] mentioned that the increased serum C-tau level in severe head injured patients was associated with compromised blood–brain barrier (severe TBI). In contrast to [14] the study reported that CSF C-tau levels and found its levels were elevated 1,000-fold in TBI patients (1,519.6 ± 3,019 ng/ml) as compared to controls (0.031 ± 0.11ng/ml). Thus, C-tau may prove to be a promising molecule in severe TBI, provided more number of studies with highly sensitive detection methods in serum and CSF are undertaken.

Regarding to Pearson correlation between GFAP level and C-tau level studied groups there was high significant (p value <0.001) and low positive correlation (R=0.402).The second novel finding of this study is that the consideration of both biomarkers together improves the sensitivity and specificity for TBI diagnosis compared with each considered alone. Both serum GFAP and C-tau levels are powerful independent predictors of TBI outcome.

The current study showed that there is a positive correlation between high serum GFAP and C-tau protein levels and early diagnosis of TBI taking all other variables in consideration. In order to determine whether there was also a positive correlation between serum C-tau protein levels and patient outcome, the present study compared the serum C-tau protein levels in patient presented with history of TBI using clinical and radiological variables to add more prediction accuracy to studied brain specific biochemical markers. The study focused on the serum GFAP and C-tau protein levels during fist 24 hours after TBI, showed the most significant differences among the three groups and thus was the most representative.

Conclusion and Recommendations

Serum GFAP level had greater value than serum C-tau in detection of intracranial lesion. There was a statistically significant but weak correlation between serum levels of C-tau and GFAP. Serum GFAP and C-tau levels in CT positive group were significantly higher compared to CT negative group. GCS had a strong significant relation with CT head findings and GFAP level. TBI biomarkers (GFAP and C-tau) have shortage in expression kinetics making them difficult (moving targets) to develop as reliable diagnostics. Blood samples used for TBI biomarkers measurement should be taken at multiple consecutive times after head trauma.

Declarations:

· **Funding:** None.

· **Acknowledgements**: None.

· **Conflict of Interest**: The authors declare that they have no competing interests.

· **Availability of data and materials:** Data will not be shared with public access.

· **Consent for publication:** Consent forms were given and signed by all subjects prior to participation

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