"Cleaved Tau Protein": A Novel Biomarker Candidate in Mild Neurotrauma in Emergency Settings

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Abstract

Background Neurotrauma has been labeled as a "silent epidemic" affecting both the developed and the developing nations. Traumatic brain injury (TBI) in humans leads to the proteolytic cleavage of tau protein called *cleaved tau* (C-tau) protein. The objectives of the study are to the role of serum cleaved tau (C-tau/τ^C) protein as a biomarker in patients with mild TBI and correlate it with the clinical progression (GCS) and clinical outcome (GOS) in emergency settings.

Materials and Methods The study has been approved by the institutional ethical committee. The study included 40 cases with mild TBI and 40 controls. C-tau protein levels were measured in venous samples in emergency using human cleaved microtubule-associated protein tau ELISA kit (by CUSABIO).

Results The mean serum C-tau protein level in cases was 44.76 $\pm$ 23.10 pg/mL (range: 12.32–96.44, 95% CI: 37.37–52.15) and controls was 33.82 $\pm$ 13.65 pg/mL, (range: 2.48–66.54, 95% CI: 29.46–38.19, $p = 0.091$). At admission the mean serum C-tau level was 65.15 $\pm$ 22.41, 43.87 $\pm$ 9.67, 26.15 $\pm$ 9.13 pg/mL in patients with GCS 13, 14, and 15, respectively. Serum cleaved tau protein levels in the good outcome group were significantly lower, that is, 40.77 $\pm$ 19.63 pg/mL (mean $\pm$ SD) (range: 12.32–88.71, 95% CI: 34.13–47.42) compared with the poor-outcome group 80.66 $\pm$ 23.10 pg/mL (mean $\pm$ SD) (range: 46.55–96.44, 95% CI: 43.88–117.43, $p = 0.004$).

Conclusion In this study, serum C-tau levels in patients with mild TBI were comparatively higher than those in the controls. Reaching a definitive conclusion will be too early and beyond the scope of this study. Thus, more studies are required in identifying its role as a diagnostic and prognostic marker in mild TBI.

Keywords
- serum cleaved tau protein
- mild traumatic brain injury
- emergency
- diagnostic
- prognostic marker

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Introduction

Traumatic brain injury (TBI), also known as *intracranial injury*, occurs when there is an external blow to the brain, which is commonly caused by vehicle accidents, bad falls, and violence. Intracranial injuries are associated with high rates of mortality and morbidity.[1] The incidence of TBI in the United States is between 180 and 250 per 100,000 per year; whereas in Europe, it is estimated to be 235 per 100,000 per year.[2,3] In India, the incidence was 160 per 100,000 per year in an epidemiological study by the National Institute of Mental Health and Neurosciences (NIMHANS) on neurotrauma.[4]

TBIs can be classified on the basis of severity as mild, moderate, and severe.[5] TBI has two phases: the primary injury occurring during impact, and the secondary injury occurring in the hours and days following the initial insult, caused by brain edema, free radical formation, or the release of inflammatory mediators, which exacerbate the initial injury by mediating cell damage or death resulting in a poor neurologic outcome.[6,7] Efforts are aimed to either prevent or reduce the secondary phase of head injury.

In the emergency settings, mainstay of treatment begins with clinical evaluation that includes Glasgow coma scale (GCS) evaluation, neurologic examination, and the imaging techniques such as computed tomography (CT) scanning and magnetic resonance imaging (MRI).[6] Till date, no single brain-specific biomarker has been unanimously accepted for routine clinical use in TBI.[8] A biomarker is an indicator of a specific biological or disease state that can be measured using samples taken from either the affected tissue or peripheral body fluids and they can be altered enzymatic activity, changes in protein expression, or posttranslational modification, altered gene expression, protein, or lipid metabolites, or a combination of these changes.[9]

Currently, biochemical markers are playing an important role in other emergency clinical settings, for example role of troponins in diagnosing and deciding the line of treatment in patients with chest pain. Thus, more research is required on similar lines to find any guiding biomarker in TBI cases.

Tau protein is a highly soluble microtubule-associated protein (MAP) associated with microtubule stabilization in neurons. It is highly expressed in thin, nonmyelinated axons of cortical interneurons, thus having distinct regional distributions in the brain, which might be helpful in determining which areas of the brain have been affected by TBI.[10] After release, this protein is proteolytically cleaved at the N- and C-terminals and diffuses into the cerebrospinal fluid (CSF) and plasma.[11] The molecular weight of C-tau in humans after TBI is 30 to 50 kDa. Monoclonal antibodies that recognize this cleaved form of tau protein used in an enzyme-linked immunosorbent assay (ELISA) format can be used to quantify serum levels of the cleaved tau protein (τ^C).[12]

Besides conventional techniques, no definitive diagnostic marker for therapeutic use to assess the severity and prognosis of TBI exists till date. Except for research purposes, no test specifically designed to diagnose TBI is available commercially. The authors chose to study cleaved tau protein as source of this 30- to 50-kDa protein are neurons and glia and lack any extracerebral source unlike S100, NSE that also have extracerebral source. Thus, C-tau is specific to central nervous system (CNS) tissue and one of the less studied biomarkers in TBI especially the cleaved form.

Therefore, newer tests are required in TBI as early as possible to ensure better patient survival and lesser long-term neurologic sequelae.

Materials and Methods

This prospective pilot study was conducted on patients presenting at the emergency department with TBI from January 2014 to July 2015. In this study, patients were included in this study as per the inclusion and exclusion criteria shown in the following text. The study was approved by the institutional ethical committee.

Inclusion Criteria

The inclusion criteria included the following: age $>$ 18 years, closed head injury, no severe coagulopathy that was defined as clinical evidence of excessive bleeding, platelet counts $<$ 1,00,000, international normalized ratio $>$ 1.4, or partial thromboplastin time $>$ 50, no clinical indication for future anticoagulation, for example life-threatening deep vein thrombi, pulmonary embolism, family, or next-of-kin available to provide written informed consent.

Exclusion Criteria

The exclusion criteria included the following: age $<$ 18 years, presented in emergency department 12 hours after injury, nonavailability of blood sample, pregnancy, patients with Alzheimer's disease, athletes, boxers, prolonged cardiac arrest at the scene of the accident, or high cervical spinal cord injury, and those who had died from uncontrollable hemorrhage or multiple life-threatening associated injuries.

Sample Size

A total of 40 patients of mild TBI were enrolled in the study from January 2014 to July 2015, along with 40 healthy volunteers from blood bank who served as the control group.

Study Protocol

Patients presenting within 12 hours of injury were included in study. Patients' demographic information was recorded at the time of enrolment. The GCS score was calculated at the time of admission, third day, and on seventh day during hospitalization. The GCS was measured on a 3 to 15 point scale with highest score reflecting normal performance. Time of injury, associated injuries and time of patient arrival at the hospital emergency, and cranial CT scan findings were recorded. The mechanism of injury was categorized as falls, assaults, motor vehicle crashes, or any other mode depending on the case. Clinical outcome was assessed with the Glasgow outcome scale (GOS). Patients were divided into two groups on basis of GOS: good-outcome group with GOS IV and V and poor-outcome group with GOS I, II, and III.

Venous blood samples from enrolled patients were collected at time of presentation into 10-mL serum separator tubes (SSTs).

After collection, the samples were allowed to clot for 2 hours at room temperature or overnight at 4°C and then centrifuged at 13,000 g for 15 minutes. The serum samples were frozen at −70° C (−94°F) and later assayed for cleaved tau (τ^C) protein using ELISA technique. Human cleaved MAP tau (C-MAPT/C-TAU) ELISA kit (BY CUSABIO-CSB-ECL013481H) available for research purposes was used in this study. A standard curve by plotting the mean absorbance for each standard on the x-axis against the concentration on the y-axis was constructed, and a best-fitting curve through the points on the graph was made and samples serum C-tau values were obtained. Patients' outcome was assessed at 6-month follow-up using GOS.

Statistical Analysis

Statistical analysis was done using SPSS 20 (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, New York). Demographic characteristics and clinical features of the patients were analyzed according to mean ± standard deviation (SD) and range (minimum-maximum). The normal distributions were analyzed applying the Kolmogorov-Smirnov test, and mean in two or more groups was then analyzed using Mann-Whitney *U* test. Furthermore, the Kruskal-Wallis test was then used to compare differences among groups followed by Tukey's test in case of significant difference. $p < 0.05$ was considered as statistically significant.

Results

In this study, 456 patients were admitted in the emergency department of neurosurgery, out of whom 146 patients had head injury. Considering the selection criteria, 40 patients of mild TBI and 40 healthy volunteers from blood bank as controls were finally included in the study (►**Table 1**).

In the study group, the mean age was 39.45 ± 15.60 years, minimum age was 18 years, and maximum age was 78 years. The mean age in the control group was 37.35 ± 12.93 years, minimum age was 18 years, and maximum age was 68 years. In this study, the mild TBI group, comprised 31 males (77.5%) and 9 females (22.5%) ($n = 40$) and the male-to-female ratio was 3.4:1. The most common mode of injury was road traffic accident (RTA) followed by assault, fall from height, fall of object on head, and sports injury. Maximum number of patients had GCS 15, followed by GCS 13 and 14. All patients who presented with history of TBI underwent routine investigations along with NCCT (noncontrast CT) head scanning. CT findings were found negative in 35% patients ($n = 14$), whereas majority of (65%) patients had positive findings viz. contusion/intraparenchymal hematomas in 35% ($n = 14$), skull bone fracture (linear and depressed) in 17.5% ($n = 7$), subarachnoid hemorrhage (SAH) in 5% ($n = 2$), subdural hemorrhage (SDH) in 5% ($n = 2$), and extradural hemorrhage (EDH) in 2.5% ($n = 1$). Approximately 90% of patients ($n = 36$) in the mild TBI group were managed conservatively. However, 10% patients ($n = 4$) in mild TBI underwent surgical intervention, depending on the type of injury.

Serum C-tau Protein

The mean serum tau protein level in the study group was 44.76 ± 23.10 pg/mL (range: 12.32–96.44, 95% confidence

Table 1 Demographic characteristics and serum C-tau protein levels

Characteristics	mild TBI cases	Control	C- tau protein level in mild TBI (mean ± SD) pg/mL	*p* Value
Male	31 (77.5%)	33	46.21 ± 23.65	$p^a = 0.544$
Female	9 (22.5%)	7	39.78 ± 21.61	
Mean age in years (range)	39.45 ± 15.60, (18–78)	37.35 ± 12.93, (18–68)		
Mechanism of injury				
RTA	25	–	50.48 ± 25.86	$p^b = 0.132$
Fall from height	3	–	52.12 ± 13.18	
Assault	9	–	29.41 ± 11.03	
Fall of heavy object on head	2	–	39.00 ± 6.42	
Sports injury	1	–	–	
GCS score				
At the time of admission (mean)	14.025	–		$p^b < 0.001$
13	15	–	65.15 ± 22.41	
14	6	–	43.87 ± 9.67	
15	16	–	26.15 ± 9.13	

Abbreviations: GCS, Glasgow coma scale; RTA, road traffic accident; SD, standard deviation; TBI, traumatic brain injury.
[a]Independent-samples Mann-Whitney U test.
[b]Independent-samples Kruskal-Wallis test.

Table 2 CT findings, GOS at discharge and serum C-tau protein levels

Results	Mild TBI cases (%)	Serum C-tau levels(Mean ± SD) pg/mL, range (max-min)	*p* Value
CT findings			
Negative CT scan	14 (35%)	26.96 ± 8.91	$p^a < 0.001$
Skull bone fracture (linear and depressed)	7 (17.5%)	41.57 ± 5.87	
Contusion/intraparenchymal hematomas	14(35%)	66.45 ± 19.31	
SDH	2 (5%)	62.63 ± 36.87	
SAH	2 (5%)	26.04 ± 4.94	
EDH	1 (2.5%)	–	
GOS at discharge			
Good outcome (GOS IV, V)	36 (90%)	40.77 ± 19.63, 12.32–88.71	$p^b = 0.004$
Poor outcome (GOS I ,II, III)	4 (10%)	80.66 ± 23.10, 46.55–96.44	

Abbreviations: CT, computed tomography; EDH, extradural hemorrhage; GOS, Glasgow outcome score; SAH, subarachnoid hemorrhage; SD, standard deviation; SDH, subdural hemorrhage; TBI, traumatic brain injury.
[a]Kruskal-Wallis test for independent samples.
[b]Mann-Whitney U test.

interval [CI]: 37.37–52.15). The mean serum tau protein level in the control group was 33.82 ± 13.65 pg/mL, (range: 2.48–66.54, 95% CI: 29.46–38.19). Compared with controls, this difference in mean serum C-tau protein was not statistically significant ($p = 0.091$). The mean serum cleaved tau protein level in males was 46.21 ± 23.65 pg/mL (range: 12.32–96.44) and in females was 39.78 ± 21.61 pg/mL (range: 12.34 ± 85.66). This difference in serum tau levels with reference to sex was, however, not significant statistically ($p = 0.54$).

The mean serum C-tau level was 65.15 ± 22.41, 43.87 ± 9.67, 26.15 ± 9.13 pg/mL in patients with GCS 13, 14, and 15, respectively, at zeroth day. Serum C-tau levels were significantly lower in patients with GCS 15 compared with in patients with GCS 14 ($p = 0.011$). Also, C-tau was significantly lower in GCS 15 as compared with GCS 13 ($p = 0.000$).

Mode of Injury and Serum C-tau Protein Levels

The mean C-tau levels in RTA cases 50.48 ± 25.86 pg/mL, assault 29.41 ± 11.03 pg/mL, fall from height 52.12 ± 13.18 pg/mL, fall of object on head 39.00 ± 6.42 pg/mL ($p = 0.132$).

Computed Tomography Findings and Serum C-tau Protein Levels

CT scans demonstrated intracranial injury in 26 patients with mild TBI. The difference between the serum tau protein values of patients with normal CT scans (26.96 ± 8.91 pg/mL) and those with evidence of injury on CT (54.36 ± 22.75 pg/mL) was statistically significant ($p < 0.001$).

Glasgow Outcome Score and Serum C-tau Levels

With reference to the outcome of the patients at discharge, serum cleaved tau protein levels within 12 hours of injury were correlated with good- and poor-outcome groups in mild TBI (►**Table 2**). In this study, 36 patients had good outcome and 4 had poor outcome.

Serum cleaved tau protein levels in the good-outcome group were significantly lower, that is, 40.77 ± 19.63 pg/mL (mean ± SD) (range: 12.32–88.71, 95% CI: 34.13–47.42) compared with the poor-outcome group 80.66 ± 23.10 pg/mL (mean ± SD) (range 46.55–96.44, 95% CI: 43.88–117.43, $p = 0.004$).

Glasgow Outcome Score at 6 Months Follow-Up

After 6 months, 32 patients had good outcome and 5 patients were lost to follow-up.

Discussion

This study was a sincere attempt to identify the role of cleaved form of an axonal protein—"tau"—in mild TBI. Various correlations of C-tau with different variables were investigated in this study. The patients were of various age groups: most patients belonged to 28- to 37-year age group (32.5%), followed by 18- to 27-year age group (22.5%). In mild TBI group ($n = 40$), the mean age was 39.45 ± 15.60 years (18–78 years). Bulut et al included 60 cases of mild TBI with the mean age of cases being 32.5 years (15–66 years).[1] Wuthisuthimethawee et al reported the mean age in their study as 34.9 ± 15.6 years (range: 15–74), focusing on minor head trauma.[13] In another study by Zemlan et al (1999), mean age was 32.4 ± 14.1 years.[12] These are studies from various geographic regions of the world, and all of them reflect that TBI is more often seen in the most productive age group that comprise a larger part of population and directly have an impact on economy of countries, as they are more involved and exposed to the daily outdoor activities. This demands more sincere efforts to be directed in finding out research molecules in TBI to reduce the mortality and morbidity. In this study, maximum patients were males (M:F ratio: 3.4:1). A review by Bruns and Hauser on epidemiology of TBI mentioned that the demographic groups at high risk for TBI

include males and individuals living in regions characterized by socioeconomic deprivation.[2]

There was no statistically significant difference in males and females with reference to serum cleaved tau levels ($p = 0.54$) in this study. The authors found no correlation of serum C-tau levels with sex in mild TBI cases in this study. Similar were the findings in other studies. In a study concerning minor head trauma, no statistically significant difference in serum tau levels in males (16.62 pg/mL [2.12–215.97 pg/mL]) and females (17.60 pg/mL [3.42–714.47 pg/ mL]) was witnessed by Kavalci et al.[14] A study concerning pediatric patients with minor head trauma conducted by Guzel et al reported serum tau protein levels to be 84.91 ± 69.30 pg/mL (5.14–367.29) and 73.0 ± 60.06 pg/mL (5.71–230.43) in male and female patients, respectively, which was insignificant statistically ($p = 0.197$).[15] Thus, various studies reported that no significant difference exist in serum tau levels with reference to males and females in TBI. This shows that serum C-tau level is a sex-independent variable in head trauma.

In this study, the most common mode of injury in cases of mild TBI was RTA, followed by assault, fall from height, fall of object on head, and sports injury. In mild head injury case, mode of injury showed no significant difference in levels of serum C-tau in this study ($p = 0.132$) (►**Fig. 1**).

In an epidemiologic study by NIMHANS, India, the most common cause of TBI was RTI (62%), followed by falls (22%), assaults (10%), and fall of objects (8%).[4] Odero et al conducted a review of RTIs and found that in developing nations, pedestrians, motorcyclists and bicyclists together were at high risk of sustaining head injuries.[16] This fits well to Indian context also but not in Western countries where motor vehicle occupants are at a greater risk compared with motorcyclists and bicyclists.

The authors found that mean serum C-tau levels (44.76 ± 23.10 pg/mL) were higher than those in the controls (33.82 ± 13.65 pg/mL) in this study, but this difference was, however, not statistically significant (►**Fig. 2**). This supports that in mild TBI, serum C-tau protein does not truly reflect the internal pathological changes in brain matter posttrauma, and for tau proteins to be released into serum from CSF through blood-brain barrier, trauma should be greater in intensity, volume, and with axonal involvement. Not many studies are available on C-tau levels in mild TBI (►**Table 3**).

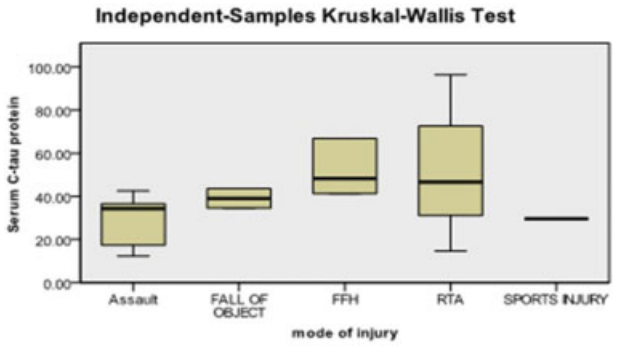
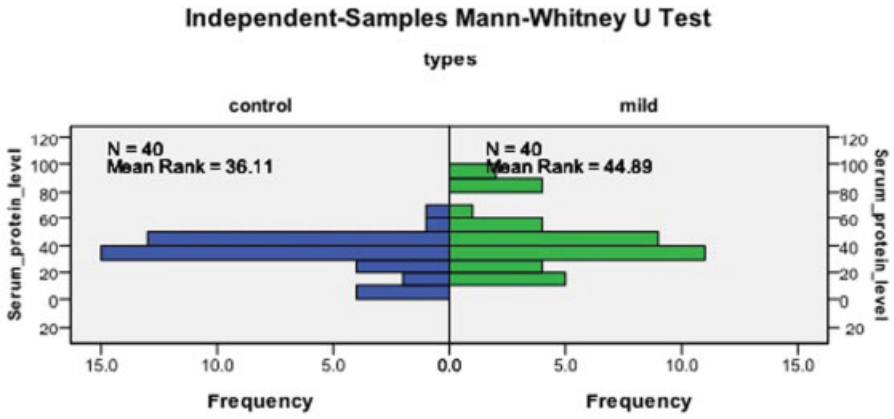


Fig. 1 Box plot showing comparison of serum C-tau protein levels and mode of injury in mild TBI ($p = 0.132$). FFH, fall from height; RTA, road traffic accident.

Mareka et al in 2008 also could not find any significant role of C-tau in serum in mild TBI. They reported that mean serum C-tau concentration was 5.02 ng/mL (SD 2.98 ng/mL). Their lowest detection limit of serum C-tau ELISA was 1.5 ng/mL. They reported that serum C-tau was not detected more frequently in all patients ($p = 0.115$) or in the subgroup with negative head CT scans ($p = 0.253$).[17] No serum cleaved tau protein could be detected by Wuthisuthimethawee et al in 2013 in either the healthy control group or the patients with mild TBI.[13] They concluded that there was no correlation of serum cleaved tau level in mild TBI, thus making it an unreliable marker in early detection and decision making in mild TBI injury patients at the emergency department. Thus, an increase in serum C-tau levels occurring after mild head trauma does not signify it to the tune of diagnostic significance. It is important to mention that C-tau protein differs slightly from tau protein. After head injury, proteolytic cleavage of tau protein weighing 48 to 68 kDa results in a truncated tau protein weighing 30 to 50 kDa and is comparatively shorter as it lacks the N- and C-terminal domains.[19] Also, different investigators have used different measurement methods; thus, these comparisons should be viewed with caution. Other studies on mild TBI are available, which detected tau protein in serum. A study on serum tau protein by Bulut et al in mild TBI measured that tau levels in serum in cases (188 ± 210 pg/mL) was not significantly higher than those in the controls (86 ± 48 pg/mL). However, in their study serum tau protein in high-risk patients (307 ± 246 pg/mL) was significantly higher than that in low-risk patients (77 ± 61 pg/mL) and controls ($p = .002$).

The mean serum C-tau level in patients with GCS 13, 14, and 15 was 65.15 ± 22.41, 43.87 ± 9.67, 26.15 ± 9.13 pg/mL, respectively, at zeroth day (►**Fig. 3**). Serum C-tau levels were significantly lower in patients with GCS 15 compared with patients with GCS 14 ($p = 0.011$). This shows that level of serum C-tau varies with the change in GCS. In this study, patients with lower GCS had significantly higher level of serum C-tau protein, which was associated with poor outcome. This difference in levels may be studied in further detail with many large studies focused on timely C-tau measurements while correlating with GCS scores. Correlating the serum C-tau levels and the GCS at which it is significant may help the authors guide further in qualitatively analyzing the significant serum tau levels and may shed some light on its role in projecting TBI prognosis. Liliang et al found in their study that the GCS score and the serum C-tau protein level were significantly associated with clinical outcome. To the contrary, Guzel et al found no statistically significant difference in serum tau protein levels according to GCS scores.

In mild TBI, mean serum C-tau level in CT-positive group was significantly higher than that in CT-negative group ($p < 0.001$) (►**Fig. 4**). These results signify that this increase in serum C-tau level may be used to discriminate between patients with and without intracranial lesions, irrespective of the severity of injury. This finding may prove to be of highly diagnostic importance in TBI in which findings may be absent in CT, but intracranial injury is present, and may differentiate between presence and absence of head injury without considering the



Total N	80
Mann-Whitney U	624.500
Wilcoxon W	1,444.500
Test Statistic	624.500
Standard Error	103.909
Standardized Test Statistic	-1.689
Asymptotic Sig. (2-sided test)	.091

Fig. 2 Comparison of serum C-tau levels in mild TBI and controls (applying Mann-Whitney U test).

severity. This may be of immense help in primary centers in identifying patients who will definitively require tertiary health care using a safe and simple blood test and at a comparatively lesser cost. Besides, it may have an important role in medicolegal cases in identifying presence or absence of head injury. Similar results were found in a pilot study by Shaw et al on adult patients with closed head injury, where the initial C-tau level of > 0 significantly correlated with a greater chance of intracranial injury on the initial CT of the head, but their study had a limitation in that it did not mentioned the actual serum C-tau levels. Contrary to these results, Kavalci et al in 2007 reported that serum tau levels increased but not significantly in patients with minor head trauma who had intracranial lesions in cranial CT (18.39 pg/mL [2.19–714.47 pg/mL]) compared with patients with negative CT findings (16.29 pg/mL [2.12–215.97 pg/mL]).[14] Bulut et al found no significant difference between the serum tau protein values of patients with normal cranial CT scans (150 $\pm$ 163 pg/mL) and of those with established disease (201 $\pm$ 223 pg/mL) ($p = 0.473$) in mild head injury. Another similar study by Guzel et al focusing on pediatric patients with minor head trauma projected that the difference in serum tau protein level in normal CT group (96.06 $\pm$ 70.36 pg/mL) as compared with positive CT group (112.04 $\pm$ 52.66 pg/mL) was not statistically significant.[15] However, these comparisons should be viewed with caution as these studies were focused on serum tau levels.

In mild TBI group, serum C-tau levels in the good-outcome group were significantly lower, that is, 40.77 $\pm$ 19.63 pg/mL, than with the poor-outcome group, that is, 80.66 $\pm$ 23.10 pg/mL ($p = 0.004$). Thus, significantly higher serum C-tau levels in patients with poor outcome indicate utility of this protein in predicting the disease prognosis. Similar promising results were also found in other studies; for example, in a study by Shaw et al, results showed that those with a serum C-tau level of > 0 were more likely to have a poor outcome, (odds ratio: 8.17, 95% CI: 1.42–47).[11] The sensitivity and specificity of serum C-tau for predicting outcome in their study was 64% (95% CI: 37–82%) and 82% (95% CI: 65–94%), respectively.

Taking into consideration the biochemical and pathophysiological changes following trauma, efforts are continuing to find diagnostic biomolecules that can reflect these in vivo pathologic changes occurring after TBI. The availability of such biomarkers may supplement currently available methods and may help quantify the extent of damage, especially in patients with closed head injury without loss of time, which is one of the most neglected factors in developing countries. Besides, availability of CT

Table 3 Studies conducted on tau protein till date focusing mild TBI

Year	Author	Study group	TBI	Control	Sample	GCS	Test	Results	Other findings
Mild head injury									
2006	Bulut et al[1]	60 adults	Mild TBI	20	Serum	Mean scale (GCS) score was 14 ± 0.6	Specific sandwich ELISA (Innotest hTAU-Ag, Innogenetics, Gent, Belgium)	S. tau levels of patients (188 ± 210 pg/mL), compared with those of controls (86 ± 48 pg/mL), not statistically significant ($p = 0.445$)	S. tau levels of high-risk patients (307 ± 246 pg/mL) were significantly higher compared with the low-risk patients (77 ± 61 pg/mL) ($p = .001$).
2007	Kavalci et al[14]	33 patients (group 2)	Minor head trauma	55 patients (group 1)	Serum	–	Not mentioned	Group 1 ($n = 55$) median serum tau protein level was 16.29 pg/mL (2.12–215.97 pg/mL) and group 2 ($n = 33$) median serum tau protein level was 18.39 pg/mL (2.19–714.47 pg/mL)	Statistical analysis revealed no significant difference between the 2 groups for tau protein values, sex, age, mechanism of trauma, and GCS score.
2008	Ma et al[17]	50 adults	Mild TBI	–	Serum	–	ELISA	τ^C is a poor predictor of PCS after mild TBI regardless of CT scan result of the head	15 patients had detectable levels of τ^C, 10 patients had abnormal findings on initial CT of the head and 22 patients had PCS.
2010	Guzel et al[15]	1–14 y-old group 1–30 patients with normal CCT findings, group 2–30 patients with intracranial lesions	Mild TBI	1–14 y-old group 3–28 control	Serum	–	Human tau immunoassay kit (BioSource International, Camarillo, California, United States) with sandwich ELISA	Mean S. tau levels: group 1 96.06 ± 70.36 pg/mL, group 2 112.04 ± 52.66 pg/mL, no statistically significant difference between the groups ($p = 0.160$).	Serum tau protein increased after minor head trauma, but concentrations are not associated with ICI. GCS score and pathologic condition in CCT were only influential variables on tau protein levels.
2013	Wuthisuthimethawee et al[13]	44 cases	Mild TBI	12 healthy volunteers (control group)	Serum	Median GCS was 15	Human Tau phosphoSerine 396 ELISA (hTau pS396) kit (BioSource International, Inc., Camarillo, California, United States).	Positive at a cutoff point of 0.1 pg/mL. Serum τC not detected in either control group or the patients with mild TBI. Serum τC level considered positive if > 0.1 pg/mL.	No correlation of serum τ^C with mild TBI was observed; proved to be an unreliable marker in early detection of and decision making in emergency.
Animal studies on tau proteins									
2001	Irazuzta et al[18]	Rats	Experimental bacterial meningitis (using type III GBS)		Brain tissue CSF, serum		C-tau sandwich ELISA quantification of serum, CSF and heat-stable tau proteins.	Whole-brain τ^C were significantly elevated in high-dose GBS inoculated animals compared with controls.	τ^C appears in S.; reflects the extent of neurologic damage. Neurobehavioral performance altered after bacterial meningitis and could be correlated with histologic and biochemical markers of neurologic sequelae.

Abbreviations: CCT, contrast-enhanced computed tomography; CSF, cerebrospinal fluid; C-tau/τ^C, cleaved tau protein; ELISA, enzyme-linked immunosorbent assay; GBS, group B *Streptococcus*; GCS, Glasgow coma scale; ICI, intracranial injury; PCS, postconcussion syndrome; TBI, traumatic brain injury.

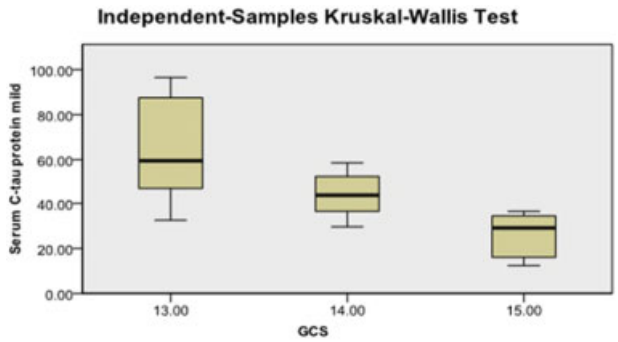


Fig. 3 Box plot showing serum C-tau protein levels in patients with mild TBI (Glasgow coma scale [GCS] 13, 14, 15).

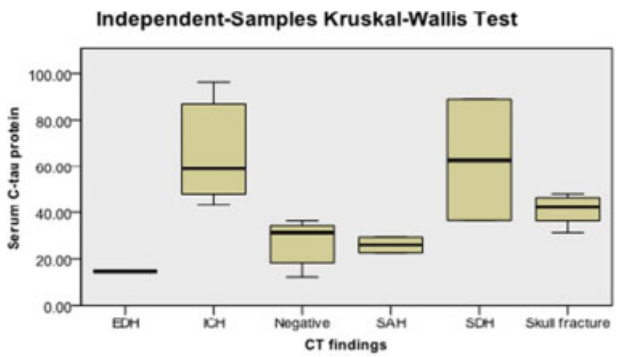


Fig. 4 Box plot showing comparison between serum C-tau levels of patients and computed tomography (CT) findings in mild TBI ($p < 0.001$). EDH, extradural hemorrhage; ICH, intracerebral hemorrhage; SAH, subarachnoid hemorrhage; SDH, subdural hemorrhage.

scans, high costs, and radiation exposure are some other factors that potentiate the need to search for other tools of early identification of TBI.

The authors observed that patients with high serum C-tau levels were found to be significantly associated with poor outcome. In mild TBI group, serum C-tau levels in the good outcome group were significantly lower than those in the poor-outcome group ($p = 0.004$). Similar promising results were also found in other studies. The degree of brain injury depends on the magnitude of injury, secondary insults, and patient's genetic and molecular response.[20]

Diagnosing TBI using clinical and radiologic parameters forms the cornerstone of treatment in the current scenario, but all have their limitations. For example, clinically, GCS cannot detect subtle neurologic signs such as latent paresis. Radiological imaging techniques such as CT scan cannot detect diffuse axonal injury, which otherwise is an important finding. Limitations of one parameter may be overcome by other parameter; thus, using combination of these parameters may increase the diagnostic and prognostic accuracy.

The sample size in this study was too small to reach a definitive conclusion. Furthermore, no measurement of CSF C-tau was done as CSF sampling was an invasive procedure. This study lacked in temporal serum and CSF C-tau measurements and its correlation that would be very beneficial in understanding the disease pathogenesis.

Conclusion

In this study, serum C-tau levels in patients with mild TBI were comparatively higher than those in the controls. Patients with lower GCS had significantly higher level of serum C-tau protein and poor outcome (GOS score), thus indicating role of tau protein in predicting the disease prognosis. However, reaching a definitive conclusion will be too early and beyond the scope of this study. Thus, more studies are required in identifying its role as a diagnostic and prognostic marker in mild TBI.

Conflict of Interest
The authors declare that they have no conflict of interest.

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Ethical Approval
The study has been approved by the institutional ethical committee (Reference No. Dean/2014–15/EC/423). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee. Patients/family member gave informed consent prior to the inclusion in the study.

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