Original Article

Diagnostic Value of Serum Glial Fibrillary Acidic Protein and S100B Serum Levels in Emergency Medicine Patients with Traumatic versus Nontraumatic Intracerebral Hemorrhage

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Abstract

Background: Glial fibrillary acidic protein (GFAP) is a brain-specific astroglial protein that is released into the blood soon after traumatic brain injury by mature astrocytes. S100B is rapidly released into the cerebrospinal fluid and bloodstream after brain damage. We compared the serum concentrations of these proteins in patients with severe head trauma (bleeding and/or fracture) or nontraumatic intracerebral hemorrhage and healthy individuals. **Materials and Methods:** The study included 63 patients (33 males and 30 females) with traumatic cerebral hemorrhage and/or cranial bone fractures or nontraumatic cerebral hemorrhage and 30 healthy control subjects. The reasons for attending the emergency department were as follows: fall from a height (n = 32), traffic accident (n = 18), nontraumatic intracerebral hemorrhage (n = 6), animal kick to the head (n = 4), and blow to the head (n = 3). **Results:** Of the 63 patients included in the study, 33 (52.4%) were male and 30 (47.6%) were female. Of the 30 healthy controls, 12 (40%) were male and 18 (60%) were female. The average age of the patients was 27 years (range, 1 month to 86 years) and the average age of the control group was 21 years (range, 18–30 years). The mean serum GFAP concentrations were 86.37 ng/mL in the patients and 38.07 ng/mL in the controls ($P < 0.05$). The mean serum S100B concentrations were 428.37 pg/mL in the patients and 103.44 pg/mL in the controls ($P < 0.05$). Eight (12.7%) patients died in the hospital; of those, the mean GCS score was 4.6, and the mean GFAP and S100B levels were 127.8 ng/mL and 860.6 pg/mL, respectively. **Conclusion:** The GFAP and S100B concentrations were significantly higher in patients with traumatic or nontraumatic brain injury than in healthy individuals, indicating that serum levels of these biomarkers may provide an alternative to computed tomography for the diagnosis of brain injury.

Keywords: *Fracture, glial fibrillary acidic protein, hemorrhage, S100B*

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Introduction

Trauma is a leading cause of death in people between the ages of 1 and 44 years, and head trauma is one of the most common reasons for emergency department visits.[1] In the United States, 1.1 million people visit the hospital annually for head trauma, and 50,000 patients die.[2] Car accidents, occupational accidents, falls from a height, gunshot injuries, and blows are the most frequent causes of head trauma.[3] Radiological monitoring is the most widely used technique for the diagnosis of head trauma in the emergency department; however, no laboratory tests are available for the diagnosis and evaluation of cerebral hemorrhage or cranial fractures. Although serum biomarkers, including creatine kinase isoenzyme

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HB and troponins, are widely used to diagnose various conditions in peripheral organs, no serum biomarkers are clinically available for the assessment of brain injury. The identification of brain-specific biomarkers is challenging, because the brain is more complex and less homogenic than other organ systems.[4] However, an increasing number of studies are focusing on the identification of serum biomarkers specific to white matter. We investigated molecules that increase in the blood following trauma-related or spontaneous brain injury, including the brain-specific astroglial protein glial fibrillary acidic protein (GFAP). Elevated serum concentrations of GFAP have been detected within 6 h of brain or spinal cord injury and decrease rapidly depending on the volume of the damage.[5] Several studies have shown that the increase in serum GFAP following brain injury is related to the pathological type and clinical outcome.[6]

S100 is a multigenic family of calcium-module proteins that are expressed exclusively in vertebrates. S100B, a member of the S100 family, is a homodimeric acidic protein consisting of two beta units with a molecular weight of 21 kDa. S100B is important for neural growth, differentiation, and regeneration. Moreover, the increase in extracellular concentration after cerebral damage suggests that S100Bplays a role in the neurodegenerative pathophysiology of damaged cells. S100B levels increase rapidly in the cerebrospinal fluid and then in the blood in response to cerebral damage. S100B levels have been shown to increase in human blood and cerebrospinal fluid in response to traumatic brain injury.[7]

We compared serum GFAP and S100B concentrations in patients who visited the emergency department with a suspected brain injury or nontraumatic intracranial hemorrhage (ICH) and healthy individuals.

Materials and Methods

Sixty-three patients with head trauma or nontraumatic ICH, who applied to emergency services and had computed head tomography scan, were included in the study. Fifty-seven (90.5%) cases were related to trauma and six (9.5%) cases were nontraumatic ICH. In total, 57 patients with head trauma were classified as having only ICH (14 patients), only cranial fractures (17 patients), and those with cranial fractures (26 patients) and cranial hemorrhage.

Inclusion criteria

- Traumatic ICH and/or cranial fracture
- Nontraumatic or spontaneous ICH, "ICH" refers to bleeding into the parenchyma of the central nervous system (including cerebrum, cerebellum, and brainstem localizations)
- Computed tomography (CT) scan taken of Emergency Department
- Volunteers who signed informed consent form
- 30 totally healthy individuals were taken as the control group.

Exclusion criteria

- Patients without computerized brain tomography
- Spontaneous subarachnoid hemorrhage
- Those who do not agree to participate in the work and do not sign the petition form.

The study was approved by the ethics committee of Adiyaman University, Faculty of Medicine, on December 20, 2016. Approximately 5 cc of blood were obtained from patients within 1 h of their arrival at the emergency department. Following hydroextraction, the supernatant serum was stored at −80°C for later use. The control group included 30 healthy volunteers with no history of cranial fracture or ICH who were in the emergency department.

Serum levels of GFAP (CSB-E08601 h; Cusabio Biotech Co., Wuhan, China) and S100B (DKO073; DiaMetra, Paciana, Italy) were measured using the quantitative sandwich enzyme immunoassay technique according to the manufacturer's instructions. Briefly, plates were incubated with standards, samples, and solutions, and then stop solution was added to all wells. Subsequently, the plates were subjected to spectrophotometric analysis at 450 nm using an EZ read 400 microplatereader (BiochromLtd., Cambridge, UK). A standard curve was drawn according to the concentrations of the standards, and the outer diameter (OD) values were calculated accordingly.

Statistical analysis

All statistical tests were performed using the Statistical Package for the Social Sciences version 15.0 (SPSS Inc., Chicago, IL, USA). All data were subjected to a one-sample Kolmogorov–Smirnov test for normality. Normally distributed variables are expressed as the mean ± standard deviation (SD), non-normally distributed variables are expressed as the median and range (min–max), and Categorical variables were compared using the Pearson Chi-square test and were expressed as counts and percentages. Comparisons among groups were assessed using independent two-sample *t*-tests or the Mann–Whitney *U*-test as suitable. Control and patient injury types were compared using the Kruskal–Wallis *H*-test. The Mann–Whitney *U*-test was used for binary comparisons. (The Bonferroni correction was applied for P values <0.008 and <0.017 [i.e., 0.05/6 and 0.05/3 comparisons].). Receiver operating characteristics of age, GFAP, S100B, and GCS were examined to identify a

cut-off value to predict traumatic ICH + Cranial fracture and traumatic cranial fracture cases. *P* value <0.05 was considered statistically significant. *P* values <0.05 were deemed to indicate statistical significance.

Results

Of the 63 patients included in the study, 33 (52.4%) were male and 30 (47.6%) were female. Of the 30 healthy controls, 12 (40%) were male and 18 (60%) were female. The average age of the patients was 27 years (range, 1 month to 86 years) and the average age of the control group was 21 years (range, 18–30 years). A fall from a height was the most frequent reason for visiting the emergency department, traffic accidents ranked second [Table 1].

Table 1: Reasons for the emergency department visit

Reason	*n* (%)
Fall from a height	32 (50.8)
Traffic accident	18 (28.6)
Nontraumatic intracerebral hemorrhage	6 (9.5)
Animal kick	4 (6.3)
Blow to the head	3 (4.8)
Total	63 (100)

The Glasgow Coma Scale (GCS) score, recorded at the initial examination on arrival at the emergency department, revealed that the brain injury was classified as mild in 45 (71.4%) patients and moderate to severe (3–12) in 18 (28.6%) patients. The mean GCS score in all patients was 12. The average duration of the emergency department visit was 45 min (range, 15–120 minutes). The mean time between symptom onset and admission to the hospital was 58.80 ± 31.97 minutes (min: 15 to max: 120). The time interval between admission to the emergency department and hospitalisation was 37.14 ± 13.25 minutes (min: 15 to max: 60 min).

Table 2: Comparison of nontraumatic intracranial hemorrhage and traumatic intracranial hemorrhage cases

Characteristicss	Nontraumatic ICH (*n*=6)	Traumatic ICH (*n*=14)	*P*	Cut off	Sensitivity 95% CI	Specitivity 95% CI	AUC 95% CI
Age (years)	69.5 (42-83)	31 (0.2-86)	0.033	55	78.57 (49.2-95.1)	83.33 (36.1-97.2)	0.804* (0.567-0.943)
GFAP$^{\Phi}$	109.80	76.47	0.207	38	0.83	0.50	0.667
S100B$^{\Phi}$	277.95 (216.8-2213.5)	251.07 (19.09-3629.8)	0.718	147	0.80	0.50	0.648
GCS$^{\Phi}$	10,5 (4-15)	12,5 (3-15)	0.841				

ICH=Intracranial hemorrhage, GFAP=Glial fibrillary acidic protein, CI=Confidence interval, AUC=Area under the ROC curve, ROC=Receiver operating characteristic, GCS: Glasgow coma scale.

Table 3: Comparison of traumatic intracranial hemorrhage + cranial fracture and traumatic cranial fracture cases

Characteristics	Hemorrhage + fracture (*n*=26)	Cranial fracture (*n*=17)	*P*	Cut off	Sensitivity 95% CI	Specitivity 95% CI	AUC 95% CI
Age	7.5 (1-79)	4 (0.1-39)	0.048	5	61.54 (40.6-79.7)	64.71 (38.4-85.7)	0.680* (0.520-0.814)
GFAP	112.65 (21.56-157.36)	66.89 (2.57-153.37)	0.004	107.5	53.85 (33.4-73.4)	94.12 (71.2-99.0)	0.765** (0.611-0.880)
S100B	305.43 (131.4-3377.9)	204.11 (124.16-364.48)	0.043	299.38	50 (29.9-70.1)	88.24 (63.5-98.2)	0.684* (0.525-0.817)
GCS	13 (3-15)	15 (10-15)	0.001	14	65.38 (44.3-82.8)	88.24 (63.5-98.2)	0.768*** (0.614-0.883)

GFAP=Glial fibrillary acidic protein, CI=Confidence interval, AUC=Area under the ROC curve, ROC=Receiver operating characteristic, GCS=Glasgow coma scale *P<0.05; **P<0.01; ***P<0.001

Table 4: Demographic characteristics and glial fibrillary acidic protein and S100B levels according to group

Characteristics	Control (*n*=30)	Patient (*n*=63)	*P*
Sex$^{\Psi}$, *n* (%)			
Male	12 (26.73)	33 (73.3)	0.264
Female	18 (37.5)	30 (62.5)	
Age (years)$^{\Phi}$	20 (18-30)	17 (1-86)	0.310
GFAP$^{\Phi}$	37.65 (8.31-64.70)	94.99 (2.57-157.36)	0.000
S100B$^{\Phi}$	96.77 (0.00-413.35)	256.93 (19.09-3629)	0.000

GFAP=Glial fibrillary acidic protein. $^{\Psi}$Pearson Chi-square test; Mann–Whitney *U*-test, expressed as the median (minimum–maximum)

Table 5: Glasgow Coma Scale score and glial fibrillary acidic protein and S100B serum levels according injury type

Characteristics	Control (*n*=30)	Traumatic and nontraumatic ICH (*n*=20)	Only cranial fracture (*n*=17)	Traumatic hemorrhage + fracture (*n*=26)	*P*[ч]
Age (years)[Φ]	20[b] (18-30)	47.5[d] (1-86)	4[b,d] (1-39)	7.5 (1-79)	0.000
GFAP[Φ]	37.66[a,c] (8.31-64.70)	78.60[a] (10.99-156.34)	66.89[e] (2.57-153.37)	112.65[c,e] (21.56-157.36)	0.002
S100B[Φ]	96.77[a,b,c] (0.0-413.35)	251.07[a] (19.09-3629.84)	204.11[b,f] (124.16-364.48)	305.43[c,f] (131.43-3377.90)	0.000
GCS score[Φ]	-	12.5[g] (3-15)	15[g,h] (10-15)	13[h] (3-15)	0.002

ICH=Intracranial hemorrhage, GFAP=Glial fibrillary acidic protein, GCS=Glasgow coma scale. [ч]Kruskal–Wallis *H*-test. [Φ]Mann–Whitney *U*-test, expressed as the median (minimum–maximum). [a,b,c,d,e,f,g]Groups with the same letter in the same line are significantly different from each other. [a]Control versus ICH (Bonferroni corrected: *P*<0.008). [b]Control versus cranial fracture (Bonferroni corrected: *P*<0.008). [c]Control versus hemorrhage+fracture (Bonferroni corrected: *P*<0.008). [d]ICH versus cranial fracture (Bonferroni corrected: *P*<0.008). [e]ICH versus hemorrhage+fracture (Bonferroni corrected: *P*<0.008). [f]Cranial fracture versus hemorrhage + fracture (Bonferroni corrected: *P*<0.008). [g]ICH versus cranial fracture (Bonferroni corrected for GCS: *P*<0.017). [h]Cranialfracture versus hemorrhage + fracture (Bonferroni corrected for GCS: *P*<0.017)

Table 6: Mean Glasgow coma scale scores and Glial fibrillary acidic protein and S100B levels according to survival status

Characteristics	Nonsurvivors (*n*=8)	Survivors (*n*=55)	*P*
GFAP[u]	128.76±34.36	80.21±41.57	0.003
S100B[Φ]	692.31 (260.7-3377.9)	229.2 (19.09-3629.8)	0.002
GCS[u]	3.62±0.91	13.21±3.11	0.000

SD=Standard deviation, GFAP=Glial fibrillary acidic protein, GCS=Glasgow coma scale. [u]Independent two-sample *t*-test (mean±SD). [Φ]Mann–Whitney *U*-test, expressed as the median (minimum–maximum)

The cranial CT scans revealed that 26 patients had traumatic ICH and cranial fracture, 14 had traumatic ICH alone, 6 had nontraumatic ICH, and 17 patients had traumatic and cranial fracture.

There was no significant difference between GFAP and S100B [$P > 0.05$, Table 2], although there was a significant difference between the age of two groups when nontraumatic ICH ($n = 6$) and traumatic ICH ($n = 14$) were compared.

Compared with patients with fractures alone ($n = 17$), the cases of patients with traumatic ICH accompanying cranial fractures ($n = 26$) were found to demonstrate significantly high levels of GFAP and S100B [$P < 0.05$, Table 3].

The mean GFAP levels were significantly higher in the patient group (94.99 ng/mL) than in the control group (37.65 ng/mL; $P < 0.05$). Similarly, the mean S100B levels were significantly higher in the patients (256.93 pg/mL) than in the controls [96.77 pg/mL; $P < 0.05$; Tables 4 and 5].

Eight (12.7%) patients died in the hospital; of those, the mean GCS score was 3.62, and the mean GFAP and S100B levels were 128.76 ng/mL and 692.31 pg/mL, respectively. Among the survivors, the mean GCS score was 13.21 and the mean serum GFAP and S100B levels were 80.21 ng/mL and 229.2 pg/mL, respectively [Table 6].

Discussion

Although head CT is the standard tool for the diagnosis and prognosis of traumatic and nontraumatic brain injury, interest in brain-specific biomarkers to assess brain injury is growing. To extend previous findings, we conducted a prospective study to investigate the usefulness of the biomarkers GFAP and S100B for the assessment of brain injury and potential clinical outcome for patients who visit the emergency department with head trauma.

After a brain injury, damaged cells release GFAP, a brain-specific astroglial protein, first into the interstitial space and then into the peripheral blood.

S100B is involved in the regulation of energy metabolism in brain cells and modulates the differentiation of neurons and glia; moreover, it is involved in several immunological functions in the brain.[8] The protein, which has a half-life of 2 h, is elevated in human blood and cerebrospinal fluid following brain injury.[8] In a recent multicenter study involving 251 patients, Welch *et al*.[9] found that the levels of GFAP (110.5 ng/mL) and S100B (2,015 pg/mL) in 36 patients with cranial hemorrhage on CT were significantly higher than those of 215 patients with normal CT findings (7.8 ng/mL and 100 pg/mL, respectively). Similarly, we found that the GFAP and S100B concentrations were significantly higher in patients with CT findings of ICH and/or fracture. We divided our patients into three groups according to CT findings: cranial bone fracture alone, ICH alone, and both ICH and cranial bone fracture. A comparison among groups revealed that the patients with ICH and cranial bone fracture had the highest serum levels of GFAP and S100B.

The astroglial protein GFAP was recently identified as a potential biological marker of intracerebral hemorrhage

in patients with symptoms of acute stroke.[10] GFAP is rapidly released in response to cell lysis caused by parenchymal hemorrhage and can be detected within 6–12 h after ischemic stroke onset; this suggests that serum GFAP can be used for the early differential diagnosis of hemorrhagic and ischemic stroke.[11] Mayer *et al*.[12] reported that the GFAP levels were elevated in patients with hemorrhagic stroke. We found six cases of spontaneous hemorrhage without trauma in our sample. Two patients with serum GFAP levels of 156 and 147 ng/mL died. Patients with low serum levels of GFAP (mean, 103.75 ± 52.49 ng/mL) survived and were discharged. These findings indicate that GFAP may be useful for diagnosis, differential diagnosis, and predicting prognosis in patients with hemorrhagic stroke.

Alpua *et al*.[7] found that the S100B levels were higher in patients with stroke than in the control group; however, they found no difference between ischemic and hemorrhagic stroke cases.[7] We found that the mean S100B concentration (594.83 ± 794.71 pg/mL) was higher in the six cases with nontraumatic hemorrhage than in the control group. Furthermore, the S100B levels were higher in the nonsurvivors. Although our findings are consistent with those reported previously, further study is needed before the clinical application of these biomarkers for the differentiation of ischemic and hemorrhagic stroke.

Lumpkins *et al*.[13] reported a relationship between elevated serum GFAP levels and mortality in a 51-year-old patient with a brain injury. We found that GFAP levels in blood samples obtained on admittance to the emergency department were higher in patients with a low GCS score. Our finding that higher GFAP levels were associated with higher mortality rates suggests that GFAP concentrations can be used to predict mortality.

In 2014, Abbasi *et al*.[14] reported that the S100B levels were high in patients who visited the emergency department with a traumatic brain injury. They found that the mean S100B concentration was significantly higher in patients with axonal damage, particularly a head fracture with ICH. Our comparison of survivors and nonsurvivors revealed that the S100B levels were significantly higher in the patients who died. Thus, S100B levels may predict mortality.

Nontraumatic ICH patients were also included in our study. The GFAP and S100B levels of patients with traumatic ICH and those with nontraumatic ICH were compared. No significant difference was found. According to the study, traumatic or nontraumatic ICH cases both increase GFAP and S100B levels similarly. Thus, the brain hemorrhage cases cannot be classified as traumatic or nontrammatic on the basis of GFAP and S100B levels. Senn *et al*. demonstrated in their study that GFAP and S100B levels increased in nontrammatic ICH patients as well.[15]

Conclusion

The measurement of serum GFAP and S100B levels in the emergency department, where most cases of head trauma are seen first, may be useful for the differential diagnosis of cranial pathologies. However, the high cost of GFAP and S100B test kits limits their use in emergency departments. Although CT scans are currently the standard diagnostic tool for head trauma in emergency departments, they expose patients to unnecessary radiation; moreover, radiologists are not on duty at all hospitals. We believe that further investigation of brain-specific biomarkers will facilitate the diagnosis of head injuries in emergency departments.

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Conflicts of interest

There are no conflicts of interest.

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