Clinical Neurology and Neurosurgery 183 (2019) 105380

Contents lists available at ScienceDirect

Clinical Neurology and Neurosurgery

journal homepage: www.elsevier.com/locate/clineuro

NRGN, S100B and GFAP levels are significantly increased in patients with structural lesions resulting from mild traumatic brain injuries

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ARTICLE INFO

Keywords:
Traumatic brain injury
Computed tomography
Glial fibrillary acidic protein
Biomarkers
Neurogranin
Calcium-binding protein S100 beta

ABSTRACT

Objective: To determine whether serum neurogranin (NRGN), glial fibrillary acidic protein (GFAP), and calcium-binding protein S100 beta (S100B) levels are associated with traumatic intracranial lesions compared to computed tomography (CT) findings of patients with mild traumatic brain injury (mTBI).
Patients and Methods: The cross-sectional study cohort included 48 patients who were admitted to the Emergency Department with a complaint of mTBI, a Glasgow Coma Scale score of 14–15, and at least one symptom of head trauma (i.e., post-traumatic amnesia, nausea or vomiting, post-traumatic seizures, persistent headache, and transient loss of consciousness). Blood samples and CT scans were obtained for all patients within 4 h of injury. Age-matched patients without intracranial traumatic pathology (CT-) were recruited as a control group. Blood samples were measured for NRGN, GFAP, and S100B levels.
Results: Of 48 patients, 24 were CT + and had significantly higher serum NRGN (5.79 vs. 2.95 ng/mL), GFAP (0.59 vs.0.36 ng/mL), and S100B (1.72 vs.0.73 μg/L) levels than those who were CT- ($p = 0.001$, $p = 0.026$, and $p < 0.001$, respectively). ROC curves showed that NRGN, GFAP, and S100B levels were sufficient to distinguish traumatic brain injury in patients with mTBI. At the cut-off value for NRGN of 1.87 ng/mL, sensivity was 83.3%, and specificity was 58.3%. At the cut-off value for GFAP of 0.23 ng/mL, sensivity was 75% and specificity was 62.5%. The optimal cut-off value for S100B was 0.47 μg/L (95.8% sensitivity and 62.5% specificity).
Conclusion: This is the first study to evaluate NRGN in human serum after mTBI. We confirmed that NRGN levels were significantly higher in CT + patients than CT- patients in the mTBI patient population. Future studies of larger populations and different age groups (especially pediatric) can help reduce the number of CT scans as a reliable and noninvasive diagnostic tool for evaluating NRGN protein levels in mTBI patients with a low probability of intracranial lesions.

1. Introduction

Mild traumatic brain injury (mTBI) accounts for 95% of all head traumas [1]. In current clinical practice, radiological imaging is the most reliable means of diagnosing mTBI. Computed tomography (CT) has led to rapid mTBI diagnosis and fewer hospitalizations, as well as unnecessary exposure to ionizing radiation [2–4]. Therefore, biomarkers for diagnosing traumatic brain injury (TBI) have been investigated in recent years. Peripheral blood analysis for brain-specific biomarkers of head trauma has shown potential clinical utility for detecting neural and axonal damage [5]. Potential candidates for brain-specific head trauma biomarkers include calcium-binding protein S100 beta (S100B), glial fibrillary acidic protein (GFAP), and the small neuronal protein neurogranin (NRGN). These biomarkers are

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https://doi.org/10.1016/j.clineuro.2019.105380
Received 10 November 2018; Received in revised form 19 May 2019; Accepted 1 June 2019
Available online 17 June 2019

synthesized by glial cells and the cerebral cortex in TBI; thus, levels are increased in the peripheral blood [6–8]. Unfortunately, in addition to increasing with head trauma, some of the identified TBI biomarkers are also accompanied by damage to non-central nervous system cells and tissues, and some of these biomarkers do not increase rapidly [9–11].

NRGN is a member of the calpactin family, which is involved in regulating postsynaptic calmodulin-Ca balance [12]. The NRGN protein is present in the cell bodies of cerebral cortex neurons in the II-VI layers and in the apical and basal dendrites of pyramidal neurons [13]. NRGN acts as a substrate for the C ɣ-isoform of protein kinase C (PKC) by binding to calmodulin [14–18]. NRGN knock-out mice are phenotypically similar to nontransgenic mice, but more likely have severely impaired learning and memory functions due to the erroneous autophosphorylation of calcium/calmodulin kinase [19]. Furthermore, NRGN has been shown to be a useful marker of neurodegenerative diseases, such as Alzheimer's, Parkinson's disease, and severe TBI [8,20,21]. In a recent study by Yang et al., serum NRGN levels were evaluated as markers of traumatic intracranial lesions in patients with severe and moderate TBI, but no study has been made to measure serum NRGN levels in mTBI patients.

The aim of this study was identify any correlations between blood NRGN, GFAP, S100B levels and abnormal cerebral CT findings in patients with mTBI.

2. Patients and methods

The cross-sectional study was limited to patients who were treated in the Emergency Department of our hospital form TBI between February 2016 and September 2016. The Glasgow coma score (GCS) for all patients was 14–15 and all presented with one or more symptoms of post-traumatic amnesia which is accepted as the presence of any elapsed time between the return of continuous memory and the accident, nausea/vomiting, post-traumatic seizure, persistent headache, and transient loss of consciousness at the time of referral to emergency service, but no injury to other tissues or organs (solid organ injury, bone fracture). Finally, 24 patients with intracranial traumatic pathology (CT+) were included as the pathological group and 24 age-matched patients without intracranial traumatic pathology (CT-). The control group was created from first admited age-matched CT negative traumatic patients who meet same criterias.

The exclusion criteria included a GCS score at hospital admission of < 14, pregnancy or possibility of pregnancy, renal failure, multiple trauma, admission to hospital > 4 h after trauma, and previous concurrent nervous system disorders. The study protocol was approved by the local Ethical Committee and written informed consent was provided from patients or parents (of patients aged < 18 years) for biological sample analyses.

Clinical and demographic variables included age, sex, length of hospital stay, cause of injury, neurological symptoms, CT scan results, and serum levels of GFAP, S100B, and NRGN. Venous blood samples were collected within the first 4 h following the trauma and 16-slice CT scans of the head without contrast enhancement were obtained (Somatom Emotion; Siemens AG, Erlangen, Germany). Epidural hemorrhage, acute subdural hemorrhage, subarachnoid hemorrhage, intracerebral hemorrhage, and cerebral contusion were accepted as CT findings of traumatic pathology. All radiological images were evaluated by a specialist radiologist blind to the patient's clinical information.

2.1. Measurement of GFAP, S100B, and NRGN levels

Venous blood samples were collected in 5-mL tubes and rapidly transported to the laboratory, where each was held at room temperature for 30 min and then centrifuged for 10 min at 3000 rpm. Serum samples were aliquoted into thirds and stored at −80 °C until analyzed. In the present study, serum protein (GFAP, S100B, and NRGN) levels were measured using protein-specific enzyme-linked immunosorbent (ELISA) kits (Human GFAP ELISA Kit, Human NRGN ELISA Kit, Human S100B ELISA Kit; Shanghai YeHuaBiological Technology Co., Ltd., Shanghai, China). The detection limits of the GFAP ELISAs were 2 pg/mL (range 0.005–2 ng/mL), NRGN ELISAs were 0.051 ng/mL (range 0.1–30 ng/mL), and S100B ELISAs were 5.03 ng/L (range 0.01–4 μg/L). The total coefficient of variation was < 10% for all assays. The biochemist and technician performing the assays were blind to the clinical and radiological findings.

2.2. Statistical analysis

The primary end point of our study was to investigate the relationship between the levels of biomarkers such as S100B, GFAP and NRGN in patients with mild head injury in the first 4 h after the trauma with abnormal traumatic CT findings. S100B and GFAP were previously evaluated by the same method in many studies. However, the relationship between NRGN blood levels and abnormal CT findings was investigated only in moderate and severe traumatic brain injury patients. For this reason, the sample size was calculated from the studies conducted for S100B and GFAP in patients with mild head injury and the number of patients was determined. Sample size was determined according to S100B and GFAP because our study is the the first study measuring NRGN levels in patients with mild head injury.

Descriptive statistics were used for continuous variables (mean, standard deviation, minimum, maximum, median). We compared two independent and normally distributed continuous variables using the Student's t-test. The parameters' diagnostic performance was assessed by receiver operating characteristic (ROC) analysis. In order to evaluate our primary end point we performed Student's *t*-test and ROC analysis. A probability (p) value of < 0.05 was considered statistically significant. All statistical analyses were performed using MedCalc software, version 12.7.7 (MedCalcSoftware, Ostend, Belgium; www.medcalc.org).

3. Results

3.1. Patient characteristics

The study population included 48 patients [38 (79%) males and 10 (21%) females] with "pure" mTBI. The demographic and clinical data of all patients are presented in Table 1. The mean patient age was 24 ± 22 (range, 5–65) years. The etiology of the patients admitted to the Emergency Department included falls in 20, assaults in eight, traffic accidents in 10, falling from elevated height in three, and other reasons in seven. Of the 48 patients, 16 presented with loss of consciousness, 11 with post-traumatic amnesia, 37 with headache, and 28 with vomiting. Of the 48 patients with mTBI, 24 were CT + and 24 were CT-. Regarding hemorrhage, 11 patients had epidural hematoma two of which was having accompanying pneumocephalus, one patient had intracerebral hematoma, two patients had subarachnoid hematoma, and three patients had subdural hematoma one of which was having accompanying subarachnoidhematoma. In addition, seven patients had cerebral contusions and 18 had cranial fractures with accompanying intracranial pathology (Table 1).

3.2. GFAP, S100B, and NRGN levels in patients with CT- and CT+mTBI

NRGN levels were significantly higher in patients who were CT+ (n = 24) than in those who were CT- (n = 24) (5.79 ± 4.14 vs. 2.95 ± 2.38 ng/mL, respectively, p = 0.001). Mean S100B levels were significantly higher in the 24 patients who were CT+ than the 24 patients who were CT- (1.72 ± 1.05 μg/L vs. 0.73 ± 0.64 μg/L, respectively, p < 0.001).The mean GFAP level was significantly higher in the 24 patients who were CT+ than in the 24 patients who were CT- (0.60 ± 0.38 vs.0.36 ± 0.25ng/mL, respectively, p = 0.026;Table 2).

Table 1
Characteristics, demographics and clinical data of study population.

Age, M (SD)	24 (22)
Sex, n (%)	
Female	10 (21)
Male	38 (79)
Cause of TBI, n (%)	
Traffic accident	10 (20.8)
Assault	8 (16.7)
Fall from height	3 (6.3)
Fall from same height	20 (41.7)
Others	7 (14.6)
Symptoms-findings, n (%)	
GCS-15	39 (81.25)
GCS-14	9(18.75)
Post-traumatic amnesia	11 (22.9)
Loss of Consciousness	16 (33.3)
Vomits	28 (58.3)
Headache	37 (77.1)
Intracranial lesion detected in CT scan, n (%)	
CT +	24 (50)
CT −	24 (50)
Epidural hematoma	9 (18.75)
Epidural hematoma + Pneumocephalus	2(4.2)
Subdural hematoma	2(4.2)
Subdural hematoma + Subaracnoid hemorrhage	1(2.1)
Subarachnoid hemorrhage	2(4.2)
İntracerebral hemorrhage	1 (2.1)
Contusion	7 (14.7)
Accompanying Skull fracture	18 (37.5)

SD, Standard Deviation; GCS, Glasgow Coma Scale, CT+, computed tomography positive scan; CT−, computed tomography negative scan.

Table 2
Description of median, mean, standard deviation, minimum and maximum of serum NRGN, GFAP and S100B levels in the different groups.

		N	Mean	Median	SD	Min.	Max.	p
NRGN (ng/mL)	**CT(+)**	24	5.79	3.87	4.14	2.06	13.04	**0.001**
	CT(-)	24	2.95	1.8	2.38	1.3	9.52	
	all pat.	48	4.37	3.64	2.11	1.3	13.04	
GFAP (ng/mL)	**CT(+)**	24	0.599	0.611	0.381	0.144	1.243	**0.026**
	CT(-)	24	0.366	0.228	0.259	0.106	1.03	
	all pat.	48	0.483	0.344	0.299	0.106	1.243	
S100B (μg/L)	**CT(+)**	24	1.72	1.83	1.05	0.37	3.37	< **0.001**
	CT(-)	24	0.73	0.43	0.64	0.22	2.60	
	all pat.	48	1.27	1.11	0.54	0.22	3.37	

3.3. Cut-off values for serum protein levels as diagnostic markers for mTBI

GFAP, S100B, and NRGN levels were evaluated in accordance with the CT findings to determine diagnostic cut-off values. Table 3 shows the individual marker area under curve (AUC) values in terms of CT- versus CT + results. Serum levels of S100B, GFAP, and NRGN on admission were significantly associated with the pathological findings on CT. The GEAP cut-off value of 0.23 ng/mL was characterized by a sensitivity of 75% [95% confidence interval (CI) = 53.3%–90.2%] and specificity of 62.5% (95% CI = 40.6%–81.2%). At the same cut-off value, the negative predictive value (NPV) was 71% and the positive predictive value (PPV) was 67%. The optimal cut-off value for S100B of 0.47 μg/L had a sensitivity of 95.8% (95% CI = 78.9%–99.9%), specificity of 62.5% (95% CI = 40.6%–81.2%), PPV of 72%, and NPV of 94%. The ROC analysis of NRGN values showed that NRGN serum levels accurately discriminated between patients with pathologic versus normal findings on CT. The optimal NGRN cut-off value for 1.87 ng/mL had a sensitivity of 83.3% (95%CI = 62.6%–95.3%), specificity of 58.3% (95% CI = 36.6%–77.9%),NPV of 78%, and PPV of 67% (Fig. 1).In addition, at an NRGN concentration of > 1.63 ng/mL, sensitivity was 100% and NPV was100%.At an NRGN concentration of > 1.95 ng/mL in the pediatric group (age, ≤ 16 years), sensitivity was

Table 3
Sensitivity, specificity, predictive values, of successive GFAP (ng/ml), NRGN (ng/ml) and S100B (μg/L) cut-off levels presence of intracranial lesions as seen on admission head CT and area under the curve (AUC).

	AUC	%95 Lower CI	%95 Upper CI	p value	Cut-off	Sensitivity	%95 Lower CI	%95 Upper CI	Specificity	%95 Lower CI	%95 Upper CI	PPV	NPV
NRGN(ng/ml)	0.767	0.635	0.899	< 0.001	1.87	83.3	62.6	95.3	58.3	36.6	77.9	66.7	77.8
GFAP(ng/ml)	0.688	0.536	0.839	0.016	0.23	75	53.3	90.2	62.5	40.6	81.2	66.7	71.4
S100B (μg/L)	0.835	0.723	0.948	< 0.001	0.47	95.8	78.9	99.9	62.5	40.6	81.2	71.9	93.7

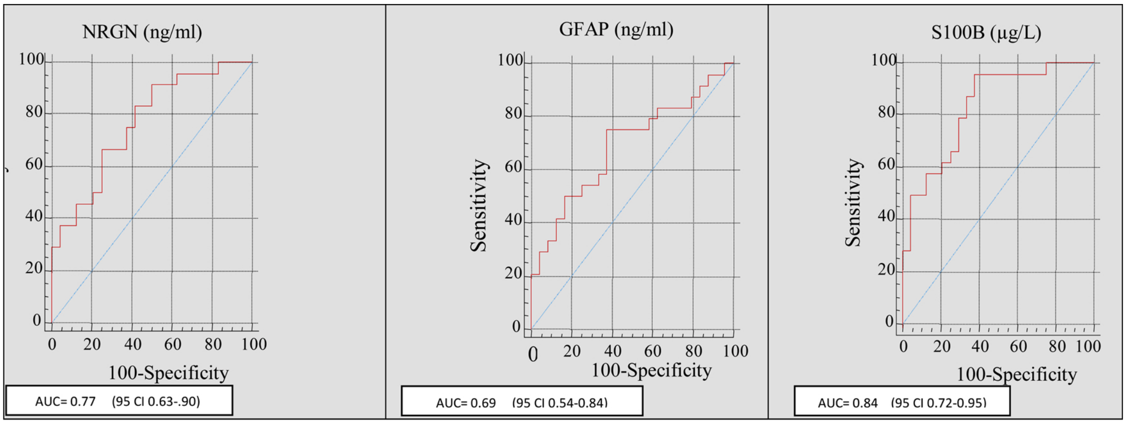

Fig. 1. Receiver operating characteristic (ROC) curve analysis CT(+) versus CT(-) patients. The area under the ROC curve (AUC) demonstrates that NRGN, GFAP, S100B levels are able discriminate between patients with and without traumatic intracranial lesions on CT.

Table 4
Contingency table of NRGN protein blood levels according to CT findings of intracranial lesions.

	age	Cut-off (ng/mL)	Sensitivity	Specificity	PPV	NPV
NRGN(ng/ml)	**4≤65 (n=48)**	> 1.63	100	17	55	100
		> 1.70	96	38	61	98
		> 1.76	92	50	65	86
	4≤16 (n=20)	> 1.95	100	70	77	100

PPV, positive predictive value; NPV, negative predictive value.

100%, specificity was 70%, and NPV was 100% (Table 4).

4. Discussion

The present study's aim was to examine peripheral blood protein levels in patients with mTBI with a GCS score of 14-15and at least one associated neurological symptom post-trauma. These results showed that serum levels of GFAP, NRGN, and S100B could be useful markers for detecting patients who have pathological CT findings of mTBI. According to our data, these three biomarkers are statistically significant for pathological CT findings of mTBI (GFAP, $p = 0.026$; S100B, $p < 0.001$; and NRGN, $p = 0.001$).

GFAP is found in white and gray brain matter and is strongly up regulated during astrogliosis. Current evidence indicates that serum GFAP might be a useful marker for various types of brain damage, from neurodegenerative disorders [22,23] to stroke [24] to severe TBI [25–30]. Hence, GFAP is a useful biomarker for assessment of brain injury, and several studies have reported its role in head trauma. Recently, serum GFAP profiles were described in severe and moderate TBI (GCS < 12) [31]. A study by Papa et al. [32] of mild, moderate, and severe TBI reported that the mean GFAP concentration in all trauma patients was 0.893 ng/mL, and the mean value in patients with a GCS of 15 was 0.531 ng/mL, which was consistent with the present study results (0.483 ng/mL). In a study of 215 patients, Okonkwo et al. showed that mean plasma GFAP level was 0.26 ng/mL in patients who were CT and 2.88 ng/mL in those who were CT+ [6]. Nevertheless, these results may be due to including patients with moderate and severe head trauma in addition to mTBI, rather than only mTBI.

In our series, the GFAP cut-off value of 0.23 ng/mL had remarkable diagnostic performance with a sensitivity of 75% and specificity of 62.5% The 0.035 ng/mL cut-off described in the literature proved to be an excellent screening tool, with 97% sensitivity and 18% specificity [32].

S100B is a neuronal survival protein that is mostly synthesized by mature astrocytes. Several studies ofS100B levels in TBI have been performed over the past 15 years. In the present study, the S100B levels in patients with mTBI were evaluated in the early period after trauma. According to our data, the optimal cutoff value for the diagnosis of mTBI is a serum S100B level of 0.47 µg/L (sensitivity, 95.8%, 95% CI = 78.9%–99.9%; specificity, 62.5%, 95% CI = 40.6%–81.2%; PPV, 72%; and NPV, 94%). In a recent study of 143 patients with a history of head injury, the inclusion criteria were the same as those used in the present investigation (i.e., GCS score of 15) [33]. Egea-Guerrero et al. [33] reported a protein S100B cut-off value of 0.23 µg/L, which identified TBI on CT with 92% sensitivity, 51% specificity, and 98% NPV, but only 20% PPV. In this last study, when the cut-off value was set to 0.23 µg/L, the results of NPV level and sensitivity were better than those in our study. More recently, Cervellin et al. [7] conducted a study of patients with mTBI using 0.38 as the S100B cut-off value, which had 100% sensitivity, 58% specificity, 100% NPV, and 54% PPV. That study's results are consistent with the present study. However, Cervelin et al. included patients with GCS scores of 14-15. Due to more severe trauma, decreased GCS scores are related to increased serum S100B levels [31].

NRGN, a small protein originally identified in the rat brain, contains 78 amino acids and has a molecular mass of 7.6 kDa [34–36]. It is found in the cell bodies of cerebral cortex neurons and in apical and basal dendrites of pyramidal neurons [37]. It is thought that NRGN can easily pass through a damaged blood–brain barrier because of its relatively small size [8]. Consequently, NRGN would be expected to be valuable for the rapid detection of brain injury. In a case-controlled study by Yang et al, using this premise, the mean NRGN level of patients with TBI was higher than that of the control group (0.18 vs. 0.002 ng/mL, respectively, $p < 0.0001$) [8]. In a study of 76 patients with TBI, the median NRGN level was 0.27 ng/mL CT + TBI patients and 0.08 ng/mL in CT(-) TBI patients [8].In the present study of 48 patients with mTBI, the median NRGN level was significantly greater in the 24 CT + patients than in the 24 CT- patients (3.8 vs. 1.8 ng/mL, respectively, $p = 0.001$). The mean values in the present study were also greater than those reported in earlier studies, which may be explained by the extended time for blood sample collection of patients with trauma in the earlier studies, (< 4 vs. < 24 h, respectively) and the age ranges of patients differed between two studies. In earlier studies, the mean patient age was 47 (range,30–56) years; in this study, the mean age was 24 (range,5–65) years. In the present study, most patients were children and adolescents. Notably, a child's brain is different from an adult's, and thus may also vary in secreted protein levels.

In our group of 48 patients, the best cut-off value for NRGN was 1.87 ng/mL, which had a sensitivity of 83.3%, a specificity of 58.3%, NPV of 78%, and PPV of 67%. At the cut-off value of 1.87 ng/mL, a high specificity is obtained, and we think that our sensitivity value is not clinically sufficient. However, 100% sensitivity, 100% NPV, and 18% specificity were obtained at the cut-off value of 1.63 ng/mL. At the same time, at the cut-off value of 1.95 ng/mL in the pediatric patient population (age, < 16 years, 10 CT +, 10 CT-), the sensitivity was 100%, NPV was 100%, and the deviation was 70%. According to the data obtained in this study a NRGN level of < 1.63 ng/mL and a S100B level of < 0.47 μg/L were unlikely or too low for CT. Of course, larger studies are needed of patients with no indication of TBI on CT images.

There are concerns about exposure to ionizing radiation as a result of unnecessary CT scans, especially in pediatric patients. In our study, NRGN was found to be a promising biomarker with good sensitivity, NPV, and specificity rates in the pediatric age group.

The GFAP and S100B results in the present study were similar to those of previous mTBI patient trials. These proteins appear to have important diagnostic sensitivities and specificities expected from a CNS protein, but have some limitations for diagnostic use in TBI patients. S100B levels in the blood are also elevated in extracranial pathologies, adipose tissue, muscle, and bone [38,39], which prevents it from being a marker specific for trauma patients. GFAP is a specific marker of intermediate filament proteins of astrocytes, and no increase in blood levels was observed without brain injury [40]. However, GFAP levels were reported to be higher in the presence of larger lesions and elevated intracranial pressure [40]. GFAP has also been detected 6 h after the onset of symptoms in hemorrhagic stroke patients, but not those with ischemic stroke [41]. This finding suggests that brain-specific markers of the lesion type or etiology may affect its release into circulation. In addition, it is possible that GFAP can be detected in the blood after injury for a few hours [8]. For these reasons, the objective diagnosis of TBI is still a matter of effort. Currently, there is no markers that have been approved by the Food and Drug Administration for diagnosing TBI. Therefore, research for accessible biological markers is still needed for the correct and reliable TBI diagnoses.

5. Conclusion

In conclusion, this study is the first to evaluate NRGN levels in human serum after mTBI. According to present study beside increased S100B and GFAP levels, NRGN levels did also increased significantly in patients with CT + m TBI. Measurement of the levels of these three molecule may help determine patient diagnosis modality. NRGN levels were better in determining the traumatic abnormal CT findings in the pediatric patient group. In future studies, larger populations of different age groups (especially pediatric) can help reduce the number of CT scans. NRGN can be a reliable and noninvasive diagnostic tool mTBI patients with a low probability of intracranial lesions.

Declaration of Competing Interest

There is no financial support. There is no conflict interest.

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