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Diagnostic values of proenkephalin and S100B protein in traumatic brain injury

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Abstract

Background: The primary aim of this study was to investigate the diagnostic values of serum S100 calcium-binding protein B (S100B) and proenkephalin (P-ENK) levels in brain damage caused by traumatic brain injury (TBI).
Methods: We prospectively collected serum blood samples of 58 adult patients admitted to our emergency department due to TBI. Serum S100B and P-ENK levels were measured and compared according to clinical findings and outcomes of the patients.
Results: When patients with brain injury were compared to controls, statistical significance was determined in both S100B and P-ENK levels. According to the receiver operating characteristic (ROC) analysis, cut-off values for serum S100B and P-ENK levels for the differential diagnosis of patients with and without brain damage were found to be 785.944 ng/mL and 2.445 ng/mL, respectively. There was a statistical significance in both S100B and P-ENK levels when patients who were discharged and those who died were compared.
Conclusions: Serum S100B and P-ENK levels are found to be elevated in patients with TBI when compared to controls. Additionally, serum levels of both markers are found to be elevated in patients with multiple lesions when compared to patients with a single lesion. Serum S100B and P-ENK levels may also be used as predictors of mortality in patients with TBI.

Keywords: emergency department; proenkephalin; S100B; traumatic brain injury.

Introduction

Trauma is the leading cause of morbidity and mortality among young populations between 1 and 44 years of age [1]. In the US, it was reported that 1.5 million people are exposed to non-lethal brain trauma, 370,000 being hospitalized and 52,000 dying, annually [2]. Currently, brain imaging methods are generally used for the diagnosis of traumatic brain injury (TBI). The search for biomarkers for predicting brain injury following head trauma still continues. Recently, concentrations of S100 calcium-binding protein B (S100B) and proenkephalin (P-ENK) in the extracellular fluid were found to increase and indicated neural damage after TBI [3]. However, studies investigating the role of these biomarkers together in TBI are rare. The aim of this clinical study was to investigate the diagnostic values of S100B and P-ENK in brain damage related with TBI.

Materials and methods

After obtaining approval from the Local Ethics Committee, 58 adult patients (>18 years) admitted to the Department of Emergency, Samsun Education and Research Hospital, due to head trauma between 1 March 2015 and 1 September 2015 were included in this prospective clinical study. On the other hand, 29 healthy volunteer subjects were grouped as controls. Written consent was obtained from each participant.

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After a detailed physical examination, computed tomography (CT) and/or magnetic resonance imaging (MRI) scans were obtained in accordance with the Canadian CT Head Rule and the New Orleans Criteria. Using radiological imaging data of the brain, the patients were divided into two groups, patients with brain injury and those without brain injury. Then, the patients with brain injury were divided into two subgroups, patients with one lesion and those with multiple lesions. Groups were compared according to clinical and laboratory findings.

The inclusion criteria were as follows: patients (>18 years) with head trauma and patients admitted to our emergency department in the first 4 h following head trauma. The exclusion criteria were as follows: pediatric patients with head trauma, pregnancy, patients under antiaggregant medication, patients with a history of neurological disease, patients with systemic diseases (hypertension, diabetes mellitus, uremia, liver disease, chronic heart failure, lung disease, etc.) and patients admitted after 4 h following head trauma.

Demographical, clinical and laboratory findings of the patients were recorded. Complete blood count (CBC) and biochemistry tests were studied from blood samples obtained on admission to the emergency department. To analyze blood S100B and P-ENK levels, venous blood samples (7 mL) were also taken. They were centrifuged at 1500 rpm for 15 min, and serum samples were stored at −80 °C until analysis. Analysis of S100B and P-ENK was performed using a Sunrise ELISA Reader (Tecan Group Ltd., Männedorf, Switzerland) in the biochemistry laboratory of Samsun Ondokuz Mayıs University.

Serum S100B levels were measured by the Human S100 calcium-binding protein B (S100B) ELISA kit (catalogue no: YHB3336Hu, Lot no: 20150618, Shanghai Yehua Biological Technology Co. Ltd., Shanghai, China). Serum P-ENK levels were measured by the Human Proenkephalin (P-ENK) ELISA kit (catalogue no: YHB2441Hu, Lot no: 20150618, Shanghai Yehua Biological Technology Co. Ltd., Shanghai, China).

Statistical analyses

All statistical analyses were made using the Statistical Package for Social Sciences (SPSS) for Windows v15.0 (SPSS, Inc, Chicago, IL, USA) software program. Data were given as arithmetic mean ± standard deviation, median (minimum-maximum) and percentages (%). Normal distribution of data was detected by Shapiro-Wilk test. Student's t-tests and the Mann-Whitney U-test (for independent samples) were used to compare the groups. For comparison of more than two groups, one-way ANOVA was used for normal distribution and the Kruskal-Wallis test was used. Pearson and Spearman's correlation analyses were used for determining correlation between variables. To determine a cut-off value for S100B and P-ENK in TBI, receiver operating characteristic (ROC) analysis was performed. A p-value >0.05 was accepted as statistically significant.

Results

Of 87 patients involved in the study, 37 were female (42.5%) and 50 were male (57.5%). The mean age of the patients was 42.7 years. There was no statistical significance according to age among the groups.

When etiologic factors of head trauma were investigated, the most common cause was determined to be beating (37.9%), followed by motor vehicle accidents (32.8%) and falling (20.7%).

In patients without brain injury, the most common complaint on admission was found to be nausea and vomiting (36.7%) and the rarest complaint was found as altered mental status (8.5%).

When patients were compared according to blood pressure (BP) findings on admission, no statistical significance could be determined.

The Glasgow Coma Scale (GCS) score of the patients varied between 6 and 15. There was a statistical significance between the patients with and without TBI according to GCS. In addition, a negative correlation between GCS and S100B/P-ENK levels was determined (S100B rho: −0.64, P-ENK rho: −0.91).

When groups were compared according to CBC and biochemistry results, no statistical significance could be determined.

The most common CT findings were found to be subdural and epidural hematoma. When subgroups (single and multiple lesions) were evaluated, S100B and P-ENK levels were significantly different (Table 1).

Table 2 shows S100B and P-ENK levels of the patients on admission to the emergency department. No statistical significance could be determined when groups with and without post-traumatic brain injury were compared according to S100B and P-ENK levels. While there was no statistical significance between patients without brain injury and controls according to S100B levels, statistical significance was determined according to P-ENK levels. However, when patients with brain injury were compared

Table 1: General characteristics of patients and controls on admission.

Characteristics	Patients with TBI (n=29)	Patients without TBI (n=29)	Controls (n=29)
Age, years	49.7±18.1	39.6±12.1	38.7±13.3
GCS[a]	8.5±1.9	13.5±0.5	15.0±0.0
SBP, mm Hg	12.7±1.4	13.3±1.6	12.9±1.5
DBP, mm Hg	7.2±1.0	6.9±0.9	7.2±0.8
Complaint on admission			
Nausea/vomiting	32%, n=10	41.4%, n=12	
Dizziness	27.6%, n=7	34.5%, n=10	
Headache	30%, n=10	17.2%, n=5	
Unconsciousness	10.3%, n=3	6.8%, n=2	
CT findings			
Subdural hematoma	17.2%, n=5		
Epidural hematoma	17.2%, n=5		
Cerebral contusion	13.8%, n=4		
Subarachnoid bleeding	10.3%, n=3		
Subdural+epidural	13.8%, n=4		
Subdural+subarachnoid	13.8%, n=4		
Subdural+contusion	10.3%, n=3		
Subarachnoid+contusion	3.4%, n=1		
Laboratory findings			
Glucose, mg/dL	145.6±22.1	149.1±22.6	139.7±22.4
AST, U/L	25.9±10.7	28.2±10.1	27.5±10.8
ALT, U/L	26.5±9.3	25.9±11.8	25.9±9.8
BUN, mg/dL	16.9±4.8	16.2±4.8	17.1±4.8
Creatine, mg/dL	0.9±0.2	0.9±0.2	0.9±0.2

[a]Statistically significant. TBI, traumatic brain injury; GCS, Glasgow Coma Scale; SBP, systolic blood pressure; DBP, diastolic blood pressure; CT, computed tomography; AST, aspartate transaminase; ALT, alanine aminotransferase; BUN, blood urea nitrogen.

Table 2: Serum S100B and proenkephalin levels according to number of lesions in patients with traumatic brain injury.

Number of lesions	Biomarker	
	S100B, ng/mL	Proenkephalin, ng/mL
Single lesion	1637.4±685	4.6±1.9
(n=19)	1469 (375.1–2558.8)	4.1 (2.5–6.8)
Multiple lesions	3184.7±338.3	8.9±1.1
(n=24)	3203.4 (2494.9–3656.4)	9.5 (7.1–9.9)
p-Value	<0.001	<0.001

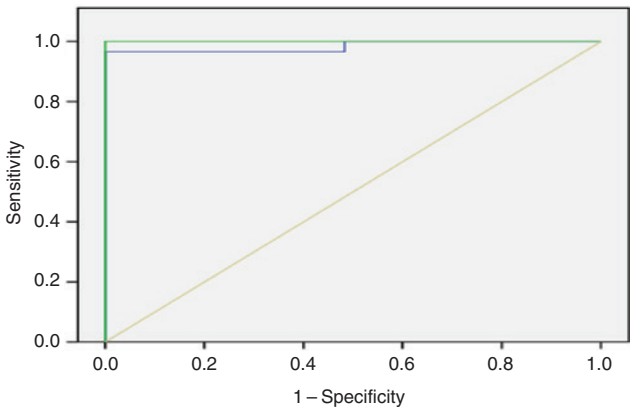

Figure 1: Receiver operating characteristic curve of serum markers in patients with traumatic brain injury.
Blue line, S100B; green line, proenkephalin; yellow line, reference line.

to controls, statistical significance was determined in both S100B and P-ENK levels.

According to the ROC analysis, cut-off values for S100B and P-ENK for the differential diagnosis of patients with and without brain damage were found to be 785.944 ng/mL and 2.445 ng/mL, respectively. Details are given in Figure 1 and Table 3.

We also determined that S100B and P-ENK levels tended to elevate as the interval between exposure to trauma and blood collection increased, and serum levels of the biomarkers tended to rise. This finding was statistically significant (p=0.013 for S100B and p=0.04 for P-ENK) (Table 4).

When the outcomes of patients with TBI were investigated, it was determined that while 45 (77.6%) were discharged from the hospital with recovery, 13 (22.4%) patients died in hospital. All of them had TBI following trauma. There was a statistical significance in both S110B

Table 3: Serum levels of S100B and proenkephalin in patients with traumatic brain injury.

Groups	S100B, ng/mL	Proenkephalin, ng/mL
Control group (n = 29)	361.9 ± 291.9	0.8 ± 0.7
	464.7 (97.9–773.1)	1.2 (0.2–1.6)
Patients without trumatic	387.8 ± 173.4	2.0 ± 0.2
brain injury (n = 29)	362.7 (95.8–656.5)	2.1 (1.6–2.4)
Patients with traumatic	2277.6 ± 956.3	6.4 ± 2.7
brain injury (n = 29)	2494.9 (375.1–3656.4)	6.7 (2.5–9.9)
p-Value	<0.001	<0.001

Table 4: Comparison of S100B and proenkephalin levels according to the interval between trauma exposure and blood sample collection.

Time interval	S100B, ng/mL	Proenkephalin, ng/mL
0–1 h[a] (n = 19)	922.5 ± 1076.6	3.3 ± 2.9
	503.4 (196.9–3656.4)	2.1 (1.6–9.9)
1–2 h[a] (n = 24)	1191.4 ± 1260.3	3.9 ± 2.9
	538.9 (95.8–3454.6)	2.2 (1.7–9.8)
2–3 h[a] (n = 10)	2097.7 ± 890.5	6.3231 ± 2.5
	2259.7 (915.4–3119.2)	6.6 (2.7–9.8)
3–4 h (n = 5)	2039.9 ± 646.6	5.1 ± 1.9
	2399.1 (1214.4–2558.8)	6.2 (2.9–6.8)

[a]Statistically significant difference.

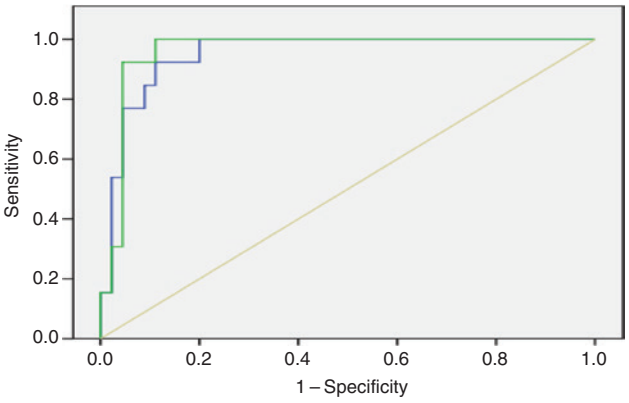

Figure 2: Receiver operating characteristic curve values of S100B and proenkephalin in patients who were discharged and who died. Blue line, S100B; green line, proenkephalin; yellow line, reference line.

Table 5: Area under the curve values of serum S100B and proenkephalin for patients who were discharged and who died.

Variables	Area under the curve	p-Value	95% confidence interval	
			Lower limit	Upper limit
Serum S100B protein	0.950	<0.001	0.897	1.004
Serum proenkephalin	0.961	<0.001	0.911	1.010

and P-ENK levels when patients who were discharged and those who died were compared. While the cut-off value for S100B in predicting mortality following TBI was found to be 1234.45 ng/mL, it was found to be 6.260 ng/mL for P-ENK. For S100B, the sensitivity in predicting mortality was 100%, the specificity was 88.9%, the positive predictive value was 72.2% and the negative predictor value was 100%. For P-ENK, the sensitivity was 100%, the specificity was 88.9%, the positive predictive value was 72.2% and the negative predictor value was 100% (Figure 2, Table 5).

Discussion

Emergency department physicians frequently face patients with trauma. In more than half of the patients, trauma is accompanied by moderate or severe brain injury. Intracranial hemorrhage (epidural/subdural hematoma, intracranial hemorrhage, contusion, etc.) is the leading cause of death in patients with TBI [2, 4]. Studies have shown that males are more frequently affected from brain damage following TBI [5, 6]. Accordingly, in our study, the majority of the patients were males. It was reported that head trauma generally occurs in patients between 35 and 44 years [3]. In a study, Jian-Bo et al. reported that the mean age of the patients with severe head trauma was 42.2 years [5]. Compatible with the literature, the mean age of the patients in our study was found to be as 42.7 years. These findings may be associated with the fact that young males tend to drive long distances at high speeds.

Gao et al. reported an association between GCS scores and mortality rates, and P-ENK [5]. In another study, it was reported that S100B was elevated in patients with minor trauma [7]. Carvellin et al. revealed a negative correlation between GCS scores of patients with TBI and S100B [8]. Goyal et al. also investigated the long-term prognosis in severe TBI and determined a negative correlation between GCS scores and S100B levels [9]. In our study, we determined a negative correlation not only between GCS and S100B, but also between GCS and P-ENK. Our findings were compatible with the literature. As P-ENK and S100B increased, GCS scores tended to decrease.

In the literature, we could not find any study investigating the relationship among blood biochemistry results (AST, ALT, BUN, glucose, creatine, etc.). In our study, we could not determine any relationship between the biochemistry results and plasma S100B and P-ENK levels.

There are studies in the literature reporting that the most common complaint in the patients admitted due to TBI is altered mental status [3, 10]. On the contrary, the results of our study revealed that the most common finding was nausea and/or vomiting. Different locations of lesions in different studies may result in conflicting results.

Lobato et al. [11] reported that the most common CT finding in patients with TBI was contusion. In another study, Dalbayrak et al. [12] reported that the most common CT finding was skull fracture. In a multi-center study, subdural hematoma was found to be the most frequent lesion in TBI [13]. In our study, epidural/subdural hematomas were found to be the most common radiological finding. Different mechanisms with varying severity of TBI may result in different findings in TBI.

The most common etiologic factor for TBI was reported to be falling from a height [3, 12]. In our study, beating was the most common cause of TBI. It was found that the etiological factors change depending of the socioeconomical status of the patients.

There are studies in the literature that show that S100B and P-ENK levels are correlated with extension of TBI and are useful in predicting the long-term prognosis in patients with TBI. Delgado et al. reported elevated S100B levels in patients with an increased intracranial blood volume [7, 13]. In our study, S100B and P-ENK levels were elevated in patients with multiple intracranial lesions. This finding was found to be compatible with the literature.

In a recent study, Sezer et al. compared blood S100B and lactate levels with CT findings. They also sub-grouped patients according to time of blood sample collection as less than and more than 3 h. They concluded that when blood samples were collected after 3 h, S100B and lactate levels were significantly higher [14]. In our study, we found that S100B and P-ENK levels tended to increase in the first 3 h of blood collection. As the time of blood collection increased (beyond 3 h), plasma levels of the biomarkers decreased. It is reasonable to obtain low plasma levels due to the half-life of biomarkers.

Yang et al. [7] reported in a study that plasma P-ENK levels were elevated in patients with intracerebral hemorrhage when compared to the normal population. Gao et al. [5] also reported that P-ENK levels were elevated in patients with head trauma. In a study, S100B was found to be elevated in patients with TBI [15]. In our study, S100B and P-ENK levels were found to be higher in patients with TBI than in patients without TBI and in the control group. When patients without TBI were compared with the controls, it was found that while serum S100B levels were not affected, P-ENK levels were elevated. Our results were compatible with the literature.

In a recent report, Sezer et al. found higher S100B levels in patients whose blood samples were collected after 3 h. They reported that this finding might be related to the half-life of S100B [14]. We also found higher levels of S100B and P-ENK as the interval between exposure to trauma and blood sample collection increased. This finding may be associated with the fact that brain injury extends as the time prolongs.

In a study by Gao et al. it was reported that P-ENK levels were higher in patients who died when compared to those who were discharged from the hospital. In their study, P-ENK levels predicted a 6-month mortality with a sensitivity of 92.1% and a specificity of 60% [5]. Accordingly, Chen et al. determined higher levels of P-ENK in patients who died when compared to those who were discharged. With a sensitivity of 90% and a specificity of 66.2%, P-ENK was found to be useful in predicting mortality in the patients with subarachnoid hemorrhage [16]. Yang et al. also reported that in the patients who died as a result of intracerebral hemorrhage, serum P-ENK levels were higher than those who survived [7]. In our study, we found that serum S100B and P-ENK levels were found to be higher in the patients who died than those who were discharged from the hospital. We also determined that sensitivity, specificity, positive predictive and negative predictive values were higher than those in the literature. These findings may result from the technique and ELISA method we used for measuring S100B and P-ENK levels.

Conclusions

Our results reveal that in patients with TBI, S100B and P-ENK levels are found to be elevated. Additionally, serum levels of both markers are found to be elevated in patients with multiple lesions when compared to patients with a single lesion. Serum S100B and P-ENK levels may also be used as predictors of mortality in patients with TBI.

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