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Evaluation of NSE and GFAP Level and Hemorrhage Volume in Terms of Prognosis in Head Trauma: A Prospective Controlled Study

Elif ÇELİKEL[1]
Ahmet Burak ERDEM[1]
Selçuk PARLAK[2]
Çiğdem YÜCEL[3]
Esra ÇIVGIN[2]
Turan TURHAN[4]
Cemil KAVALCI[5]
Muhammed Evvah KARAKILIÇ[6]
Berkin KARAPINARLI[7]

Abstract: The diagnosis process and follow-up of patients who come to the emergency department with head trauma are important for physicians. We examined the usability of biomarkers released with increased volume due to intracranial hemorrhage in emergency services. 120 cases admitted to our hospital with blunt head trauma between 2017-1018 were included. GCS, blood NSE and GFAB levels, radiological bleeding volume, and patient outcomes were analyzed. When we analyzed NSE and GFAP levels, GCS and bleeding volume, we found a statistically significant slight, negative correlation. We interpreted that when GCS is calculated low, NSE and GFAP increase and this increases the suspicion of intracranial hemorrhage clinically. NSE and GFAP levels are good in predicting intracranial bleeding. Intracranial bleeding can be interpreted by looking at the levels of these biomarkers.

Keywords: head trauma, intracranial, hemorragic volume

Kafa Travmasında NSE ve GFAP Düzeyi ve Kanama Hacminin Prognoz Açısından Değerlendirilmesi: Prospektif Kontrollü Bir Çalışma

Özet: Acil servise kafa travması ile gelen hastaların tanı süreci ve takibi hekimler için önemlidir. Acil servislerde intrakraniyal kanama nedeniyle artan hacimle salınan biyobelirteçlerin kullanılabilirliğini inceledik.2017-1018 yılları arasında hastanemize künt kafa travması ile başvuran 120 olgu dahil edildi. GKS, kan NSE ve GFAB

[1] Corresponding author, MD, Department of Emergency, Ankara City Hospital, Ankara, Türkiye, drelifkaya@gmail.com, 0000-0001-5597-7757
[1] MD, Department of Emergency, Ankara City Hospital, Ankara,Türkiye, drabe0182@gmail.com. 0000-0002-3618-6252
[2] Ass. Prof, Department of Radiology, Ankara City Hospital, Ankara,Türkiye, selcukparlakdr@gmail.com 0000-0002-3318-7399
[3] MD, Department of Clinical Biochemistry, Gülhane Training and Research Hospital, Ankara, Türkiye, yucelcidgdem80@gmail.com, 0000-0003-2647-440X
[2] MD, Department of Radiology, Ankara City Hospital, Ankara, Türkiye. esrayurduseven@gmail.com. 0000-0003-4790-3146
[4] Prof, Department of Clinical Biochemistry, Ankara City Hospital, Ankara,Türkiye, amcaturhan@gmail.com, 0000-0002-7484-0681
[5] Prof,Department of Emergency, Antalya Training and Research Hospital, Antalya, Türkiye, cemkavalci@yahoo.com, 0000-0003-2529-2946
[6] Prof, Department of Emergency, Osmangazi University, Medicine School, Eskişehir, Türkiye, evvahka@gmail.com. 0000-0003-1414-8914
[7] MD, Department of Emergency, Marmaris State Hospital, Muğla, Türkiye, berkinkarapinarli@gmail.com. 0000-0003-2401-8793

seviyeleri, radyolojik kanama hacmi ve hasta sonuçları analiz edildi. NSE ve GFAP düzeylerini, GKS'yi ve kanama hacmini analiz ettiğimizde, istatistiksel olarak anlamlı, hafif, negatif bir korelasyon bulduk. GKS düşük hesaplandığında NSE ve GFAP'nin arttığını ve bunun klinik olarak kafa içi kanama şüphesini artırdığını yorumladık. NSE ve GFAP seviyeleri kafa içi kanamayı öngörmede iyidir. Bu biyobelirteçlerin seviyelerine bakılarak kafa içi kanama yorumlanabilir.

Anahtar kelimeler: kafa travması, kafa içi, hemorajik hacim

INTRODUCTION

Traumatic brain injury (TBI) is a condition that might cause serious morbidity and mortality all over the world (Taylor et al., 2017). Furthermore, TBI can result in an acquired disability, leading to psychological problems and thus aggravating care costs (Thelin et al., 2019). Different types of hemorrhages can occur after head trauma alone or together and including subarachnoid hemorrhage (SAH), subdural hemorrhage (SH), epidural hemorrhage (EH), or parenchymal hemorrhage (PH). Following up these patients in the emergency department is challenging due to their neurological conditions. The Glasgow Coma Score (GCS) is commonly used to determine the neurological status of TBI patients. However, GCS might be inaccurate in some cases such as circadian rhythm, sedation, poisoning, and miscommunication (Teasdale et al., 2014). Computerized tomography (CT) scan is used for radiological examination in TBI patients. However, CT scans are costly and pose a risk of radiation exposure (Fayngersh & Passero, 2009). As an alternative to CT, the use of different biomarkers has been investigated in recent years due to the ease of examination and low cost for TBI patients. Neuron-specific enolase (NSE) and glial fibrillary acidic protein (GFAP) are frequently investigated among many TBI biomarkers (Zetterberg et al., 2013). NSE is a glycolytic enzyme that increases following cell injury in the central and peripheral neurons (Papa et al., 2008). NSE can be found in the blood as 0-12.5 ng/mL in healthy individuals (Mehta et al., 2022). GFAP is a protein of astrocytes and can be measured in peripheral blood after TBI within a few hours (Vos et al., 2004). GFAP can be found as 0.15-0.49 ng/ml in healthy individuals (Nylen et al., 2006). It has been shown that the use of biomarkers in TBI patients can reduce CT scanning and therefore its possible side effects and provide important information about the diagnosis, risk stratification and management of the patient in the emergency department (Bazarina et al., 2018; Aydın et al, 2018). In addition, biomarkers can be used for monitoring patients with a possibility of recurrent hemorrhage (Duda et al., 2020; Mrozek et al., 2014). Because of these features, we aimed to evaluate the usability of NSE and GFAP levels in TBI patients.

MATERIALS and METHODS

This study was designed as a prospective case-control study. After the approval of Numune Training and Research Hospital Ethics Committee. The data was collected between 01.01.2017 and 01.01.2018 from patients who had blunt head trauma. Our study was carried out in accordance with the Declaration of Helsinki rules. An informed consent form was obtained from the patients included in the study or their relatives. Patients over the age of 18 with blunt head trauma who volunteered in this study were included in the study. Collision with a hard object, traffic accident, falling from a height, and battering were considered as blunt head trauma. The patients with traumatic intracranial hemorrhage (TIH) were named as group A and the patients without traumatic intracranial hemorrhage were named as group B. Group B patients served as the control group of the study. Group A was divided into 4 groups: epidural hemorrhage group (EG), subdural hemorrhage group (SG), parenchymal hemorrhage group (PG), and multiple hemorrhage group (MG). Patients with SAH did not included any group because we were not able to quantify their hemorrhagic volume. GCS, age, trauma mechanism, intracranial hemorrhage types and volumes, blood NSE and GFAP values, and survival, intensive care, and death

records of the patients were recorded. The relationship between blood NSE and GFAP levels of Group A and Group B was examined. To assess the poor prognosis of the subgroups, the relationship between the blood NSE and GFAP levels of the patients who died and were discharged was analyzed.

Sample Collection

Blood samples were taken from the patients intravenously into a red-capped biochemistry tube (Beckton Dickinson) in the first 6 hours and were centrifuged at 1500 g for 10 minutes. Separated sera were aliquoted into Eppendorf tubes and stored at -80 °C until the time of analysis. Serum levels of NSE and GFAP were detected with commercial Enzyme-Linked Immunosorbent Assay (double antibody sandwich ELISA method) test kits (DiaMetra Italy, and Biovendor, Czech Republic respectively) according to the manufacturer's protocol. Both parameters are presented as ng/ml. Intra-assay and inter-assay coefficient of variation (CV) for NSE were 4.4 % and 11.2%, while CV for GFAP was 3.8% and 6.1%, respectively. Reference range for NSE is 0 - 12.5 ng/ml and for GFAP is 0.05 - 150 ng/ml. CT examinations were performed using a Toshiba Aquilion 64 (Toshiba Medical, Tochigi, Japan) scanner with a 1.5 mm slice thickness. A digital Workstation (TeraRecon Aquarius Workstations, Terarecon, CA, USA) was used for the analysis of images and three-dimensional (3D) volume measurements. CT evaluations were completed by two expert radiologists by consensus. Since the subarachnoid hemorrhage could not be detected by the measurement method, it was not included in the subgroups.

Definition

GCS is a scale that evaluates the level of consciousness of patients with head trauma. Patients score the lowest 3 and the highest 15 points (Table 1).

Table 1. Glaskow coma score (GCS).

Eye opening	Motor response	Verbal response
Spontaneous 4	Obeying 6	Oriented 5
To speech 3	Localizing 5	Confused 4
To pain 2	Withdrawal 4	İn appropriate 3
None 1	Flexing 3	İn compehensible 2
	Extending 2	None 1
	None 1	

Outcome

Patients who were followed up and discharged were accepted as a good prognostic indicator. Admission to intensive care, need for surgery, and mortality was evaluated as poor outcomes. The main outcome of our study is that the increasing in NSE and GFAP values synchronous with decreasing in the GCS value in intracranial hemorrhages.

Statistical Analysis

All statistical analyses were carried out using IBM SPSS (version 23.0; IBM Corp, Armonk, NY). Descriptive statistics were indicated in frequencies within lesion and trauma. To reveal the relationship between NSE, GFAP levels and GCS, lesion volume, and patients' last status, a Pearson correlation test was performed. Statistical significance was evaluated by using a two-tail t-distribution table with a 95% confidence interval (NSE, lower bound: 16.74, upper bound: 24.63; GFAP, lower bound: 3.89, upper bound: 9.63). The levels of NSE and GFAP were compared between the control and hemorrhagic group by using an independent *t*-test. Data are reported as mean ± standard deviation unless stated otherwise, and $P < 0.05$ was considered significant.

RESULTS

A total of 120 cases were included in this study. Group A = 53 cases, (45 male, 8 female), mean age 56; Group B = 67 cases (50 male, 17 female), mean age 52. The data are summarized in Table 2.

Table 2. General data.

	Gruop A	**Gruop B**
Age	Mean 56	Mean 59
Gender	Female n:8 Male n:45	Female n:15 Male n:52
Trauma	Fall %11.8 (n:22) Traffic accident %9.1(n:17) Beaten %7.5 (n:14)	Fall %37.31 (n:25) Traffic accident %34.32 (n:23) Beaten %28.35 (n:19)
GCS	Mean 12.73	Mean 15
NSE	Mean 20.68	Mean 9.94
GFAP	Mean 6.75	Mean 8.22
Hospital outcome	Discharged %79.24 (n:42) ICU %39.62 (n:21) Operation %23.40 (n:11) Ex %9.43 (n:5)	Discharged (n:67)

We detected the highest number of cases with 35.84% (n = 19) aged ≥ 70 years and above. The most frequent trauma mechanism was fall at 11.8% (n = 22). Frequency rate of hemorrhage seen in Group A was isolated SAH at 43.39% (n = 23), while SH was 18.86% (n = 10), PH was 3.77% (n = 2), and EH was 7.5% (n = 4) and 26.41% (n = 14) MH. In addition, extremity or thoracic trauma was seen as accompanying trauma in 14.89% (n = 7) cases. In Group A Pearson correlation was run to determine the relationship between NSE with GFAP level depending on hemorrhagic volume, GCS, type of hemorrhage, and patient outcome. There was a mild, negative correlation between NSE with hemorrhagic volume and GCS, which was statistically significant (r = -.284, n = 53, P = .039 and r = -.401, n = 53, p = .003, respectively). Furthermore only a mild, negative correlation between GFAP and GCS, which was statistically significant (r = -.386, n = 53, P = .004). We did not find significant differences between hemorrhage volume and NSE and GFAP levels in our patients with SH (P = 0.09 and P = 0.082, respectively). In MHG group, we found a weak negative relationship between NSE and volume (r = -0.28), and a weak positive relationship (r = 0.17) between GFAP and volume (Table 3).

Table 3. Mean values of volume, GCS, NSE, and GFAP according to bleeding types.

Bleeding types	**GCS**	**Volume (cm^3)**	**NSE (ng/ml)**	**GFAP (ng/ml)**	**Volume-NSE**	**Volume-GFAP**
Subdural (n:10)	14.5	28	15.83	6.96	P:0.90	P:0.82
Epidural (n:4)	15	31.55	18.71	2.94	P:0.20	P:0.60
Parenchymal (n:2)	14.5	11.44	10.56	12.94	-	-
2 and more hemorrhage (n:14)	10.64	20.34	21.85	9.11	P:0.334	P:0.573
SAH (n:23)	12.69	0	22.64	5.03	-	-

We performed the Pearson correlation and we observed a negative moderate relationship between GCS-NSE (P = 0.008, r = -0.36), and a stronger negative correlation (P < 0.0001, r = -0.51) was found in the GCS-GFAP relationship (Table 4).

Table 4. Comparison of NSE, GFAP levels and GCS.

	Mean	GCS
GCS	13.13	-
NSE	20.68	P: .008
GFAP	6.75	P: .000

In the ROC analysis, we compared the NSE and GFAP values in Groups A and B. The cutoff is 10.31 for NSE and 2.54 for GFAP. We found a statistically significant ($P < 0.05$) NSE (Figure 1).

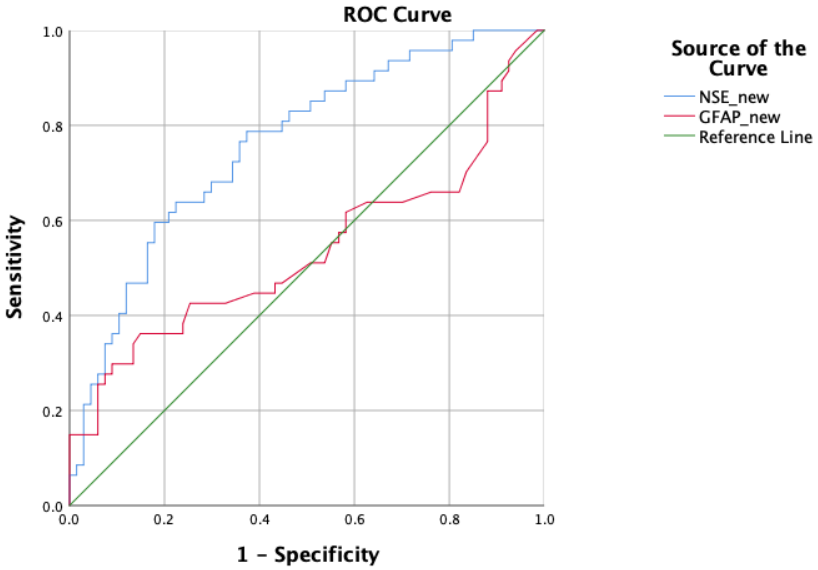

Figure 1. Roc analysis.

DISCUSSION

This study was conducted to demonstrate the clinical utility of NSE and GFAP in patients with TBI. ICHs' may cause increased intracranial pressure, resulting in increased mortality or poor outcome (Tito et al., 2018; Salihoviç et al., 2013). In a study on ICH hemorrhage volume determined that epidural ≥30 ml/cm^3, and subdural ≥10 ml/cm^3 levels for large ICH. While medical follow-up is at the forefront in small ICH, there are different opinions about the treatment strategy to be followed in moderate and large hemorrhages. It has been stated that the neurological examination findings are effective in the treatment to be chosen in cases with moderate-to-large or advanced hemorrhage (Castellanos et al., 2005). GFAP is found in gray and white matter and is known to be released when astrocytes are damaged (Diren et al., 2020). The increase in GFAP level was found to be more specific in head trauma cases and it was stated that it could be used as an early indicator for brain damage (Pelinka et al., 2004; Duchen, 1984; Sharguie et al., 2020). Another study showed that the mortality rate was found to be higher in trauma cases with increased GFAP levels (Vos et al., 2010). NSE is released as a result of damage to axons. NSE and GFAP values were higher in Group A. NSE had more predictive power in detecting ICH, therefore, NSE value can be used to predict hemorrhage in head traumas. In a study on NSE, a significant increase was observed after head trauma and an inverse relationship was found between NSE and GCS values in patients with moderate to severe head trauma (Meriç et al., 2010; Cheng et al., 2014; Miao et al., 2020). In our study, the correlation we found between GCS and GFAP in our study was stronger than NSE. This situation was compatible with the literature. At this point, we think that GFAP levels can be used as an alternative to GCS in consciousness monitoring. When we compared the GFAP values of the patients with SAH with the SHG and MHG, we could not obtain significant

results. But when we look at NSE, we found a significant relationship between SAH and the MG. NSE levels were higher in our SAH patients compared to other groups. It was observed that NSE increased more in SAH and GFAP increased more in PG. We think that the increase in NSE value in SAH is due to the higher probability of axonal injury compare to other types of hemorrhage. SAH was seen in our 5 cases for ex, NSE was 21.54 ng/ml and GFAP was 14.43 ng/ml, which was higher than the surviving patients. There has been a significant increase in cases with an ex.

In this study, we examined the relationship between the volume of hemorrhage and NSE-GFAP level but we could not find a significant relationship.

CONCLUSION

NSE and GFAP can be used to predict ICH in TBI. While NSE was stronger in ICH, GFAP was found to be stronger in GCS follow-up. In addition to radiological imaging, biomarker levels can be checked in patients with minor head trauma who do not have a history of medicinal use and have no neurological findings. In cases where radiological examinations are limited, such as pregnant and pediatric patients, NSE and GFAP levels can be used to evaluate the outcome, follow-up, and discharge status. It can be used for larger prospective studies in terms of NSE and GFAP follow-up parameters in pediatric patients.

Limitation

Because the number of our patients was small, the distribution among hemorrhage types was not balanced. Our mortality rate was low in the patients we included in our study. This caused difficulties in our comparison with survivors. We could not look at the volume relationship between all hemorrhage groups and NSE-GFAP because the volume of SAH measurement could not be performed.

Conflict of Interest: There is no conflict of interest among the authors.

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