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The Diagnostic Competence of Glial Fibrillary Acidic Protein in Mild Traumatic Brain Injury and Its Prognostic Value in Patient Recovery

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ABSTRACT

AIM: To show whether the glial fibrillary acidic protein (GFAP) levels are significantly higher in the serum of patients with mild traumatic brain injury or not.

MATERIAL and METHODS: The level of serum GFAP was measured in 176 patients suffering from brain trauma. The ability of GFAP in predicting the presence of intracranial lesions and the need for neurosurgical intervention was analyzed using the area under the receiver (AUC) operating characteristic (ROC). By passing three months from mild TBI, the Post-Concussion Symptoms Questionnaire (PCSQ) as well as the physical and mental evaluations were performed using the SF-36 questionnaire.

RESULTS: Of 176 patients included, 79.5% had no complications and symptoms by passing three months from traumatic brain injury. The AUC for GFAP was 72.6%, which revealed a good accuracy in predicting the need for neurosurgical intervention.

CONCLUSION: GFAP, as a predictive factor in people with mild TBI diagnosis who need neurosurgical operation, expressed a favorable diagnostic effect.

KEYWORDS: GFAP, Neurosurgery, Traumatic brain injury, Mild TBI

INTRODUCTION

Head is the most common site of injury, and head trauma is known as the most common cause of hospitalization and the leading cause of death in trauma patients. Traumatic Intracerebral hemorrhages can be divided into primary and delayed hemorrhages. Accordingly, primary hemorrhage refers to a group receiving evidence of bleeding in the CT scan that is taken up to the first 6 hours after the trauma. In this regard, Delayed Traumatic Intracranial Hematoma (DITCH) shows no evidence of hematoma in the initial CT scan, and hematoma is sown in the last CT scan, which is taken after 6 hours of trauma due to various reasons. Based on the scale of the initial Glasgow coma scale (GCS), Traumatic Brain Injury (TBI) is traditionally divided into three categories as follows: mild, moderate, and severe. Simultaneously, the diagnostic and therapeutic challenges of TBI is often more significant, especially in mild cases. In the clinical assessments and literature conducted on TBI, the mild TBI is less studied, because the initial symptoms are non-specific and subjective, and have overlap with psychological disorders. Also, many patients with mild TBI entirely recover, and mortality and severe disability are relatively rare in these cases (7,9,23).

Microglia are the small, non-neuronal, and mesodermal origins of interstitial cells that form a protective part of the central nervous system, which make up 5 to 20 percent of the total population of glial cells. Accordingly, these cells are known as the first population of central nervous system cells responsible for brain damage, infections, and inflammatory reactions such as Alzheimer's disease, multiple sclerosis (MS), encephalopathy, cerebral glioma, and spontaneous subarachnoid hemorrhage (3). In this regard, glial fibrillary

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acidic protein (GFAP) is a protein encoded by the GFAP gene in human beings. During its development, this type III intermediate filament expressed by most of the abundant cells of the central nervous system (CNS), including astrocytes and epidermal cells (21,25,29).

One approach to this problem is identifying biomarkers that can be measured at the acute stage of TBI that can be beneficial in treatment guiding as well as medication management. Most of the studies in this field have just focused on moderate to severe, even though the clinical potential of biomarker discovery seems to be higher in mild TBI.

A search on the related literatures revealed that few studies have examined GFAP for mild TBI. Therefore, this paper attempted to show whether the GFAP levels are significantly higher in the serum of patients with mild traumatic brain injury compared to the control group. Moreover, the validation of GFAP was discussed in the diagnosis of intracranial damage in these patients. This study was designed to answer whether GFAP levels are significantly higher in the serum of patients with a mild traumatic brain injury compared to that of the control group. Moreover, this study aimed to consider the effectiveness of GFAP and the possibility of replacing this biomarker with brain CT scan.

MATERIALS and METHOD

This study was a prospective epidemiological analysis, and the study population included the patients with TBI who were referred to the two trauma hospitals in the southwest of Iran in 2019". The Inclusion Criteria were the age of 16 years old and over, clinical diagnosis of TBI, those who have indications for brain CT scan in terms of the National Institute for Clinical Excellence Criteria (NICE), and less than 6 hours elapsed between the event and examination. Finally, the GCS was 15-13 (mild TBI). The exclusion criteria included the age of less than 16 years old, explosive or penetrating damage, chronic subdural hematoma, previous brain disorders, TBI requiring no CT, and living in another province that makes it difficult to follow-up.

In the current experiment, the level of serum GFAP was measured in 176 patients suffering from brain trauma, having 13-15 GCS, and the need for brain CT in terms of the NICE criteria. The blood samples were taken from the participants to evaluate the GFAP biomarker at the time of entering in to this study, which were then centrifuged (1,000 × g, 10min) at 4°C. The plasma was immediately frozen on dry ice and stored at -70 until the time of experiment (17). Subsequent analysis was performed using a BIOTECH laboratory kit by applying the chemiluminescent immunoassay sandwich method. Notably, in this study, the laboratory technicians were blinded to the clinical data and CT scan results.

Positive results of CT scan included acute epidural or subdural hematoma, cortical contusion, ventricular compression, ventricular trapping, cerebral herniation, intraventricular hemorrhage, hydrocephalus, subarachnoid hemorrhage, cerebral edema, post-traumatic ischemia, intracranial hematoma, and Cerebral venous sinus thrombosis (CVST). Moreover, the assessment was performed by passing three months from mild TBI using the Post-Concussion Symptoms Questionnaire (PCS) as well as performing the physical and mental evaluations using the SF-36 questionnaire (2,28).

Statistical Analysis

Descriptive statistics (mean, frequencies, and proportion) were used to describe the study variables. Biomarker level was considered as a continuous variable, and Logarithmic transformations were performed on data that were not normally distributed. Differences between groups were tested using an independent sample t-test for continuous variables and a chi-square test for categorical variables. The ability of GFAP to predict the presence of intracranial lesions and the need for neurosurgical intervention were analyzed using the area under the receiver operating characteristic (ROC) curve (AUC). Moreover, the AUC of 1.0 was considered as very well and below 0.5 as weak. Classification performance was measured by sensitivity, specificity, positive, and negative predictive values with a 95% confidence interval (CI). Also, statistical significance level was set at $p<0.05$.

RESULTS

This study included 176 patients who were referred to two reference hospitals due to traumatic brain injury. The mean age of the patients under study was 36.4 ± 16 years old, the youngest subject was 16 years old, and the oldest one was 90 years old**.** The average length of time between injury and the check-up by a physician was 50.4 ± 13.8 (minimum 30′ and maximum of 1 hour). The mean serum level of GFAP measured using the laboratory kit was 2.67 ± 2.12 ng / mL with a minimum of 0.1 and a maximum of 16.4. In addition, GCS of patients had a minimum of 14 and a maximum of 15 based on the observation and examination. Also, none of the patients had a previous history of substance abuse. Of 176 patients included, 141 were men (80.1%), and 35 (19.9%) were women. The participants of the present study were examined for the presence or the absence of underlying diseases such as hypertension, heart disease, and diabetes, which 6.82% of clients had at least one of the above-mentioned underlying diseases. The participants of this study were divided into three groups of accidents (131 people), direct trauma (25 people), and falls (20 people), based on the reason of traumatic brain injury and referring to the above-mentioned medical centers. Accordingly, the most common cause of traumatic brain injury was accident.

The results of the CT scan in the patients under study were found to be normal for 168 individuals and positive for 8 individuals with intracranial trauma-related lesions. Among those with positive CT scans, surgery was performed on four patients with intracranial lesions. In fact, of 176 patients, the surgery was performed for 2.27%.

In the present study, 50 patients (28.4%) had no side effects and symptoms after traumatic brain injury, and 126 patients (71.6%) had different complications, as discussed below (Table I).

Furthermore, of 176 patients who participated in this study, 140 patients (79.5%) had no complications and symptoms by passing three months from traumatic brain injury. Moreover, 36 people (20.5%) had different complications, according to the RPCS questionnaire (Table II).

SF-36 is a 36-item questionnaire in 8 subscales. Correspondingly, these eight areas can be assembled into two summary scales as follows: the physical component score and the mental component score. Each scale is scored in terms of a standardized scoring protocol ranged from 0 to 100, where higher scores display a better recovery status. The mean score of quality of life in each one of the subscales of the questionnaire is separately displayed in two groups of patients with normal and abnormal CT SCANs. Moreover, the mean difference was calculated in each subscale, and no significant difference was observed between these two groups with normal and abnormal CT SCANs in each one of the subscales (Table III).

The quantitative variables studied in the two groups of patients based on the CT scan revealed that, serum levels of GFAP and age were significantly associated with CT scan response, and other cases did not have these factors (Table IV).

Of 176 patients examined, eight patients with positive CT SCAN diagnosis had brain lesions, and 168 remained patients had no brain lesions. Also, in this study, the ROC curve was used to determine the diagnostic value of the GFAP in intracranial lesions. In this regard, the area under the ROC curve represents the diagnostic accuracy of the GFAP serum level. Besides, it shows that the AUC (area under the curve) is 42.5% (73.5% -11.5% CI: 95%), which means that the accuracy of this test is unacceptable in the determination of brain lesions. According to the results, the GFAP serum level of 1.35 ng/ml with 50% sensitivity and 44% specificity is the optimal cut-point for detection of intracranial lesions. (High accuracy of 90% is excellent, the accuracy of 90 -70% is good, the accuracy of 70 -50% is acceptable, and the accuracy of less than 50% is unacceptable) (Figure 1).

Of 176 patients, four patients underwent surgery. The ROC curve and AUC (area under the curve) indicated the diagnostic

Table I: Symptoms in the Subjects at the Time of Referral and Immediately After the Injury

Post-traumatic symptoms	Have symptoms n (%)	No symptoms n (%)
Headache	74 (42)	102 (58)
Vomit	61 (34.7)	115 (65.3)
Amnesia	6 (3.4)	170 (96.6)
↓ LOC	16 (9.1)	160 (90.9)
Vertigo	28 (15.9)	148 (84.1)
Nausea	42 (26.9)	134 (76.1)

Table II: Symptoms in the Subjects Three Months After Injury is Based on the RPCS Questionnaire

Concussion symptoms after three months	Have symptoms n (%)	No symptoms n (%)
Fatigue	1 (0.6)	175 (99.4)
Headache	14 (8)	162 (92)
Dizziness	5 (2.8)	171 (97.2)
Forgetfulness	2 (1.1)	174 (98.9)
Irritability	10 (5.7)	166 (94.3)
Sleep disturbance	15 (8.5)	161 (91.5)

Table III: Quality of life Based on the SF-36 Questionnaire and CT SCAN Report

Sub-Scales	Mean ±SEM		p
	Abnormal CT SCAN	Normal CT SCAN	
Physical functioning	85 ± 0.00	84.8 ± 1.7	0.69
Mental health	63 ± 6.7	64.5 ± 7.2	0.57
Social health	65.6 ± 14.6	59.6 ± 11.6	0.16
Physical role	93.7 ± 17.8	92.4 ± 14.8	0.8
Emotional role	95.8 ± 11.8	96.4 ± 10.3	0.87
Vitality	68.7 ± 5.8	65.1 ± 6.5	0.13
Pain	67.1 ± 10.8	64.5 ± 10.5	0.48
General health	71.2 ± 5.8	69.4 ± 90.1	0.57
Physical component score	78.8 ± 6.7	77.7 ± 6.0	0.60
Mental component score	73.3 ± 7.0	71.4 ± 5.9	0.38

**The statistically significant level of P-value is less than 0.05.*

Table IV: Quantitative Variables Studied in Two Groups of Patients, Based on CT SCAN Report

Variables	Mean ±SEM		p
	Abnormal CT SCAN	Normal CT SCAN	
Age	64.78 ± 20.3	35.04 ± 14.5	**0.0001**
Time (hours) *	0.81 ± 0.25	0.84 ± 0.23	0.72
GFAP (ng / mL)	4.27 ± 6.17	2.02 ± 2.37	**0.019**
SF-36 score	77.06 ± 5.64	76.14 ± 4.44	0.57

** The statistically significant level of P-value is less than 0.05.*

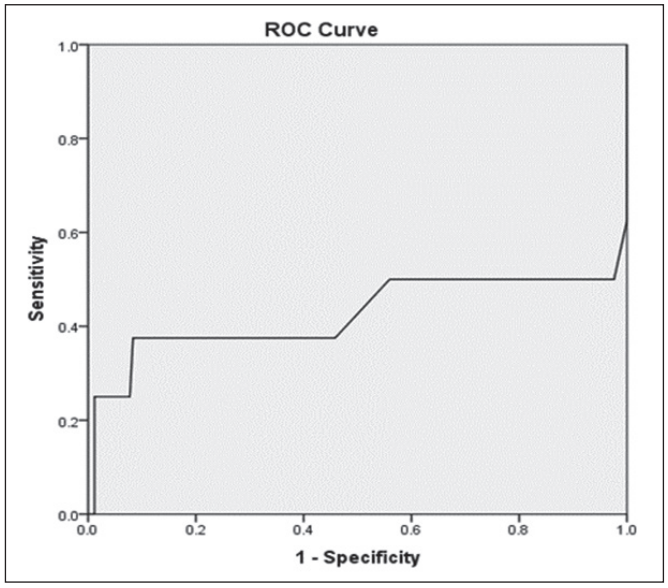

Figure 1: Receiver operating characteristic curves for GFAP to distinguishing between the presence and absence of intracranial lesions on CT scan. AUC 42.5% (73.5% - 11/5% CI: 95%).

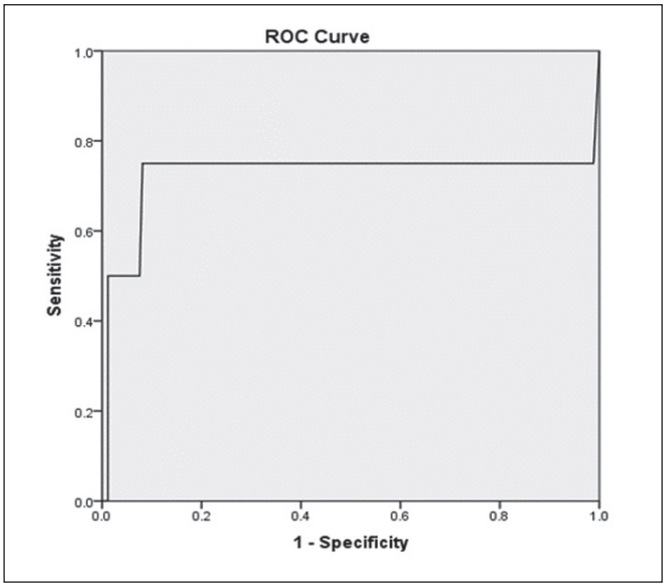

Figure 2: Receiver operating characteristic curves for GFAP to Predicting the need for neurosurgical intervention.

accuracy of the GFAP in the prediction of the need for neurosurgical intervention in patients with traumatic brain injury. In this frame, the AUC was 72.6% (31.7% -7%): CI: 95%), which showed a good serum GFAP level accuracy in predicting the need for neurosurgical intervention in patients with traumatic brain injury. According to the results, the GFAP serum level of 3.9 ng / Ml with 75% sensitivity and 92% specificity is the optimal cut-point to predict the need for surgical intervention (Figure 2).

DISCUSSION

Serum GFAP can be used as a marker in various brain injuries, including neurodegenerative disorders (16), strokes (20), and severe TBI (1). Therefore, various studies, including the present study, have examined the GFAP in mild TBI. Similar to the present study, in several epidemiological studies, it was shown that the percentage of male patients diagnosed with TBI was higher than female patients, and an increase can be seen in the proportion of men in almost all age groups. (Except those over 75 years old who have an approximately equal percentage of men and women). However, it can be said that the high rate of men was varied in these studies (6,13,15).

The brain hurt and TBI can be very varied, and in different societies and cultures, the amount of TBI types (mild, moderate, and severe) greatly varies. However, some studies have reported that 90-70% of traumatic brain injuries are mild (mTBI), which can be concussive or non-concussive (12). Mild TBI may have some symptoms such as fatigue, headache, vertigo, irritability. Due to the transient nature of these physiological symptoms, some of the long-term consequences of mTBI may be overlooked. There is a possibility that brain injuries may be invisible on CT scans and MRIs (8). So, this is principally important for people with recurrent minor brain injuries like athletes, because these primary injuries can cause secondary disruptive processes that disrupt nerve tissue and the brain's feedback pathways in several biological pathways. Therefore, it seems necessary to measure some markers in mild to moderate injuries (4). In this regard, those symptoms that do not go away within three months of recovery from trauma have the potential to impair a person's ability either

in performing daily activities or returning to work (22). In the present study, 20% of the patients still had the glory of trauma after three months, most of which included insomnia, nervousness, and headaches, and in fewer cases, vertigo and attention deficit hyperactivity disorder were reported. Based on SF-36, participants' quality of life indicates that the most common problems after three months of traumatic injury were the decreased energy and fatigue, social health, emotional role, and pain. Also, a similar study found that after three months of trauma, approximately 80% of participants had no symptoms, about 22% reported fatigue at work, and 17 % reported inability in maintaining the needed standards at work (5). An emotional and physical symptom self-reporting showed that, after mTBI, the cognitive impairment after three months was often associated with other mental, emotional, and physical criteria (24). According to some results, post-traumatic stress disorder, SF-36, and quality of life are related to some factors such as age at the time of trauma, gender, duration of trauma, mental and physical symptoms. Furthermore, the patient's physical condition after the TBI is known as a strong predictor of physical and mental health (26).

As shown in the results section, the diagnostic value of the GFAP for intracranial lesions with CT scans based on the ROC diagram was unacceptable. Whereas, after examining the relevance between GFAP and the need for neurosurgical intervention, the area under the ROC chart showed the excellent accuracy of this biomarker. Correspondingly, in line with these findings, Yue et al. stated that GFAP could be used as a commercial biomarker for intracranial lesions observed on MRI, but they are not visible on CT scans (30).

In the present study, the mean level of GFAP was 2.67 ± 2.12 ng/ml, and the maximum and minimum values were 16.4 and 0.1 ng / ml, respectively. In this regard, different studies have reported different average values for GFAP. Mayer et al., in 2013, showed a concentration of 0.07± 0.11 mg/ml for GFAP in healthy individuals (14), and Lei et al. in 2015 reported an average amount of 0.058 ng/ml for GFAP in healthy individuals (11). Moreover, Papa et al. reported that the mean GFAP concentration in all trauma patients was 0.893 ng/ml, and the average amount in these patients with GCS = 15 was 3.0531 ng/ml (18). Thus, different amounts of GFAP have been reported in similar articles, which were not in agreement with the current study work.

The biomarker kinetics is essential because its expression can change time-dependently. Exogenous degeneration, for example, may begin a few minutes after mTBI; however, it may be continued for weeks and months (27). The secretion peak of biomarkers can be affected by various factors that are not even related to the severity of the injury. In this regard, some non-traumatic factors can be named as dehydration, blood pressure, ambient temperature, and the patient's sleep status. Also, other studies noted several limitations such as low blood sample size and CSF, differences in severity of the injury, differences in patient cohort profiles, differences in control group profiles, and other aspects of the study, all of which can impair the correct understanding of biomarker kinetics (31). Thus, performing significant and frequent studies with higher accuracy are needed to understand this issue.

Another critical point is the source of biomarker secretion. The blood-brain barrier and the lymphatic system are two pathways, in which proteins are transferred from the brain to the systemic circulation. An increase in biomarker number in the blood can be considered due to various reasons such as disruption of the blood-brain barrier, changes in the glymphatic system, the increased expression of biomarkers in the brain, or a combination of the above three, that are due to the very variable nature of TBI. Therefore, depending on the type and severity of TBI, different factors can affect the oscillations of biomarkers (8,10,19).

CONCLUSION

In the present study, GFAP, as a predictive factor in people with mild TBI diagnosis who need neurological surgery, expressed a favorable diagnostic effect. The difference among the results of different studies can be due to differences in skull pathology or different sampling or a combination of the two. In this regard, many studies have suggested further standardization of TBI.

Confirming the results of the present study require performing more detailed studies with more samples, so that more reliable indicators can be organized for serum biomarkers to reflect clinical disorders or even their prognosis.

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