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The Association of Serum Glial Fibrillary Acidic Protein Level, and Outcome of Traumatic Brain Injury

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Abstract

Currently, the initial evaluation of patients with traumatic brain injury depends on examining the Glasgow coma scale (GCS) and imaging. But both modalities have limitations. Several studies have focused on biomarkers in traumatic brain injury because biomarkers are easily measured. A biomarker often used to assess the outcome and severity of traumatic brain injury is serum glial fibrillary acidic protein (GFAP). This study was aimed to find the association between serum GFAP levels with the outcome of traumatic brain injury. In 60 subjects with TBI recruited, serum, taken at hospital admission, was analyzed for GFAP. Data collected were the severity of injury based on the GCS. One month later, the outcome was assessed based on the Glasgow outcome scale (GOS). Besides GFAP, some variables that affect the outcome, such as age, pupillary reaction, therapy, hypotension, were also analyzed. There was a highly significant association between serum GFAP levels on admission with outcome one month after onset ($p < 0.001$). Multivariable analysis showed that GFAP was the strongest in predicting unfavourable outcomes. The higher serum GFAP contribute to unfavourable outcomes of traumatic brain injury.

Keywords: Biomarker, traumatic brain injury, glial fibrillary acidic protein, outcome

A. INTRODUCTION

Head injuries are the highest contributor to death and disability in young adults and are expected to be the leading cause of death and disability by 2020, with an annual incidence of 1.7 million and >50,000 deaths and 5.3 million people with severe disability (Roozenbeek et al. ., 2013; Neher et al., 2014). Head injury is often considered a silent epidemic because patients are exposed to unclear (incidental) risks and lack of public awareness of the dangers of this head injury (Schouthen & Maas, 2011; Farooqui, 2011; Begaz, 2013; Yokobori et al., 2013).

Traumatic Brain Injury (TBI) is a disorder of the brain caused by a mechanical force from outside the body that can cause abnormalities in the

cognitive, physical, and psychosocial aspects of a person temporarily or permanently and are associated with reduced or disturbed status of a person's consciousness.

TBI can result in a variety of cognitive, physical, and psychological impairments that can have a significant impact on an individual's life.

Traumatic Brain Injury occurs when an external force injures the brain, such as a fall, sports injury, motor vehicle accident, or explosion, resulting in loss of consciousness or memory loss. Injuries can occur as a consequence of a direct hit to the head or as a result of the acceleration or deceleration of brain tissue, which results in brain injury as a result of an internal impact with the skull. Both of these methods have the potential to result in tissue injury, edema, inflammation, and internal bleeding.

Traumatic Brain Injury can cause systemic secondary brain injury that will worsen the patient's condition, such as hypoxia, hypotension, hyperpyrexia, hyperglycemia, seizures, and electrolyte disturbances. Early detection and appropriate treatment of TBI patients with electrolyte disturbances will improve neurological status and reduce morbidity and mortality.

Recent clinical studies have explored the use of biomarkers as diagnostic and prognostic tools (Schiff et al., 2012). These biomarkers originate from multiple cells in the central nervous system (CNS), can be examined rapidly and repeatedly, and describe structural damage associated with brain injury (Pelinka, 2004; Yokobori et al., 2013). One of the frequently used biomarkers is glial fibrillary acidic protein (GFAP).

Glial fibrillary acidic protein (GFAP) is a unique biomarker; it is an intermediate filament released into the blood following TBI and is found only on glial cells in the central nervous system (CNS) (Andersson H, 2012). Astrocytes undergo astrogliosis in response to brain injury, which is characterized by a significant increase in the number of glial cells, astrocyte hypertrophy, and GFAP intermediate filament buildup. Numerous studies have established GFAP as a diagnostic and predictive marker in traumatic brain injury. The diagnostic function is based on GFAP's capacity to distinguish between patients with and without brain injury. Interestingly, GFAP levels were normal in patients undergoing polytrauma without sustaining a TBI, confirming GFAP's status as a brain-specific biomarker (Schiff et al., 2012).

Numerous studies have demonstrated a correlation between elevated GFAP levels and the severity and outcome of TBI, implying that GFAP can be used to provide predictive information for patients with TBI. The Glasgow Outcome Scale is used to determine outcomes (GOS). The cut-off value for GFAP was derived using Receiver Operating Curves (ROC) from prior investigations (Schiff et al., 2012).

Nylen et al. (2007) investigated the association of elevated GFAP levels with poor outcomes. GFAP levels detected in 98% of Severe TBI patients exceeded the reference GFAP levels (<0.15 mcg/l) with a median value ten times greater than the reference value. GFAP levels reach their peak 1-2 days after TBI and return to normal within 1-2 weeks (Schiff et al., 2012).

Lumpkin et al. (2008) conducted a study on TBI patients and showed that GFAP is a strong predictor of mortality in TBI. Using a cut-off point of 0.001 ng/dl, the specificity of GFAP reaches 100% and sensitivity of 50-60% (Schiff et al., 2012).

Several further researchers compared GFAP to other biomarkers, including S-100B and Neuron Specific Enolase (NSE). Pelinka et al. studied 92 individuals who had sustained a serious TBI and discovered that GFAP and S100B were significant predictors of mortality (AUC GFAP=0.84, AUC S100B=0.78) (Schiff et al., 2012). Vos et al. (2010) examined GFAP, S100B, and NSE levels in 85 patients with traumatic brain injury. The AUC±SE values for these three biomarkers indicated an increase in patients with severe TBI (GFAP=79.40.05, NSE=78.20.06, S100B=67.70.05). GFAP showed a sensitivity of 85 percent and a specificity of 52 percent when used with a cut-off of

1.5 ng/ml. GFAP has an 80% sensitivity and a 59% specificity for predicting a poor result. Multivariate research revealed that GFAP was the strongest predictor of worse outcomes following TBI (Schiff et al., 2012).

In contrast to other studies, Wiesmann et al. (2010), an observational cohort study examining the comparison of GFAP and S100B levels, found that the correlation between GFAP levels at admission and outcome after 6 months was not significant ($r = 0.40$; $p > 0.05$).

Research on GFAP in TBI in Indonesia is still very limited. Sriyanto's research (2007) examined association between an increase in epidural hematoma volume (EDH) and an increase in plasma GFAP titer and obtained significant results ($p=0.000$, correlation coefficient 0.992). Sinulingga conducted the latest study in 2014, which analyzed GFAP levels with severity of TBI at Adam Malik General Hospital Medan and obtained significant results ($p = 0.0001$).

Although the TBI of GFAP levels with TBI outcomes has been proven, the predictive value of biomarkers in predicting outcomes is still not strong enough. Thus, it still needs further validation.

B. METHOD

The purpose of this observational cohort study was to examine the association between serum glial fibrillary acidic protein (GFAP) levels and the outcome of TBI. The study took place between December 2014 and December 2015. The research was conducted at the Emergency Department of Dr RSUP. M. Djamil Padang and the Biomedical Laboratory of Andalas University's Faculty of Medicine.

The group consists of people with TBI who present to the hospital's Emergency Room (IGD) Dr. M. Djamil Padang. The sample for this study was drawn from all people who met the inclusion criteria for TBI. Patients who met the inclusion criteria were chosen using a sequential sampling technique. Patients who met the research's inclusion criteria were enrolled immediately as study subjects. Patients with TBI who presented to the hospital's Emergency Department satisfied the inclusion criteria for the study by having onset within 48 hours, being between the ages of 18 and 60, and being willing to participate. Patients who have had a stroke in the past, central nervous system tumour, status epilepticus, central nervous system infection, sepsis, multiple sclerosis, open TBI, spinal cord injury, and dropout for any reason were not included in the study. The minimum sample size estimate is calculated based on the sample size formula for observational studies, hypothesis testing on the relative risk (Madiyono et al., 2002) and the total sample size is 30 people.

TBI patients were obtained from anamnesis, both auto and or also heteroanamnesis. The history includes the cause of the TBI (traffic accident, domestic violence, falls, fights, etc.), the onset of the TBI (within hours), the presence of vomiting, fainting or dizziness shortly after the incident, alcohol and drug consumption shortly before the injury. Headaches, previous history of alcohol and drug consumption. History of stroke, central nervous system infections, CNS tumours, and multiple sclerosis.

The emergency room physician assessed the physical examination of TBI at DR M. Djamil Hospital and researchers using the Glasgow coma scale (GCS), whose assessment includes: (E)ye opening, (M)motoric response, (V)verbal response.

Supportive tests for TBI include laboratory tests. Laboratory examinations include serum GFAP levels, routine haematological examinations and other clinical chemistry. GFAP examination was performed once when the patient was admitted. The sample is serum derived from venous blood without anticoagulant, the same as clinical chemistry examination. All laboratory tests were carried out at the Biomedical Laboratory of the Faculty of Medicine, Andalas University. For blood samples without anticoagulants immediately processed into the serum. The remaining serum was put into an Eppendorf tube and stored in a freezer at –80°C (pooling) until GFAP analysis was performed to maintain

efficiency immediately after clinical chemistry examination. All sample tubes were labelled with the research subject's identity. The procedure for examining serum GFAP levels was using the ELISA method.

The subjects in this study were managed following the treatment for TBI patients in the Emergency Installation of Dr. M.Djamil Hospital, Padang. The tools used are ELISA Reader, serum taking device: vacutainer without anticoagulant 3 cc, spluit 3 cc, alcohol swab, dry cotton, tourniquet, and GFAP Reagent Kit.

For categorical variables, the results are reported as percentages; for continuous variables, the results are expressed as mean (SD) or median (minimum-maximum), depending on whether the data have a normal or aberrant distribution. Patient demographic data is displayed in the form of frequency distribution data. The basic characteristics of the research subjects included the number of subjects, gender, age, onset, length of stay, pupillary reactions, hypotension, presence or absence of surgery.

To determine the association between GFAP levels and the outcome of TBI, the study sample was divided into two groups based on the cut-off point directly determined from this study, namely the high GFAP group and the normal GFAP group (GFAP < 2, 92 ng/ml). The statistical test used to determine the association between serum GFAP levels and the outcome was the Chi-square test or Fisher exact, Kolmogorov Smirnov as an alternative to obtaining the Relative Risk (RR) value. P-value < 0.05 was considered statistically significant. Factors that play a role in determining the outcome were also analyzed, such as age, therapy is given, pupillary reaction, and hypotensive conditions. If among these factors had a significant association with the outcome ($p<0.25$), a multivariate analysis (logistical regression) was performed to determine which variables had the most influence on the outcome of TBI. Statistical analysis was carried out with a computerized system.

C. RESULT AND DISCUSSION

To ascertain the connection between serum glial fibrillary acidic protein levels and the outcome of TBI a prospective cohort observational study was done. The number of research subjects was 60 people. The basic characteristics of research subjects can be seen in tables 1 and 2.

1. Univariate Analysis

Univariate analysis is a technique for studying data on a single variable in isolation; each variable is studied independently of the others. In this analysis, there is only one reliable variable. The results of the univariate analysis in this study are presented in the following table:

Table 1. Univariate Analysis of Basic Characteristics of Research Subjects

Variable	Score
Gender, n (%)	
Male	43 (71.7)
Female	17 (28.3)
Age, year, median (min-max)	27.5 (18-60)
TBI mechanism, n (%)	
Traffic accident	58 (96.7)
Fall	2 (3.3)
Length of stay, days, median (min-max)	3 (0-30)
Onset, hour, median (min-maks)	6.5 (1-24)
Pupil reaction, n (%)	
Negative	11 (18.3)
Positive	49 (81.7)
Hypotension, n (%)	
Yes	4 (8.3)
No	55 (91.7)

Therapy, n (%)	
Conservative	58 (96.7)
Operative	2 (3.3)

Source: data proceed

Based on the data in table 1 above, it can be seen that the majority of respondents are male, which is 43%, while the majority of TBI mechanisms occur due to traffic accidents (58%) while the rest are due to other accident factors such as falls. Most pupil reactions were no pupillary reactions, as many as 49 people or approximately (81.7%).

Furthermore, namely 55 respondents or about 91.7%. And the last variable is filled by the therapy variable, divided into two types of therapy, namely conservative therapy and operative therapy. Most respondents used conservative type therapy, which amounted to 58 people or around 96.7%).

2. Bivariate Analysis

Bivariate analysis is one type of analysis used with the condition of the number of two variables. This analysis is relatively simple but can produce very useful test results. This analysis can also be understood to determine the association between two variables. In this type of analysis, two measurements are made for each observation; the samples used can be paired or each independent with its treatment. The results of the bivariate analysis in this study are presented in the following table:

Table 2. Bivariate Analysis of Basic Characteristics of Research Subjects

Variable	Outcome		p
	Bad, n=23	Good, n=37	
Gender, n (%)			
• Male	14 (60.9)	29 (78.4)	0.143[a]
• Female	9 (39.1)	8 (21.6)	
Head injury mechanism			
• Traffic accident	21 (91.3)	37 (100)	0.143 [b]
• Fall	2 (8.7)	0 (0)	
Length of stay, days, median	1 (1-2)	4(1-30)	0.001 [c]
Onset, hour, median	6 (1-24)	7 (1-24)	0.248 [c]
Age, median, (min-max)	36 (18-60)	23 (18-56)	0.009[a]
• 18-45	13 (56.5)	32 (86.5)	RR= 2.308
• 46-60	10 (43.5)	5 (13.5)	
Hypotension			
• Yes	5 (21.7)	0 (0)	0.006[b]
• No	18 (78.3)	37 (100)	RR= 3.056
Therapy			
• Operative	1 (4.3)	1 (2.7)	1.000[b]
• Conservative	22 (93.7)	36 (97.3)	
Pupil reaction			
• Positive	12 (78.3)	37 (100)	0.000[b]
• Negative	11 (21.7)	0 (0)	RR= 4.083

Information: a: Chi-square; b: Fisher's exact; c: Uji Mann Whitney

The test carried out in table 2 above is the Mann-Whitney test, which determines the significance of the differences between the two populations. The data scale used is the ordinal scale. Furthermore, the levels of GFAP in TBI patients are presented in Table 3 below:

Table 3. GFAP levels in TBI patients

Variable	Score
GFAP levels, ng/ml, median (min-max)	2.48 (0.49-40.12)

Source: data proceed

GFAP acts as a scaffolding protein for the astrocyte cytoskeleton. As an astroglial protein, GFAP is required for the astrocytic process to maintain its shape and motility. As an astroglial protein, GFAP is required for the astrocytic process to maintain its shape and motility. Additionally, glial fibrillary acidic protein contributes to white matter architecture, myelination processes, and the blood-brain barrier's stability. According to the literature, GFAP has a high degree of brain specificity, as no extracerebral sources for this protein have been found. GFAP is not released by astroglial cells under normal physiological settings, and there are no measurable amounts of GFAP in the blood serum of healthy persons. The release of GFAP from brain tissue into the circulation is crucial because it signifies the loss of astrocytic structural integrity due to cell necrosis and mechanical disruption, as well as the disintegration of the blood-brain barrier structure. The following table illustrates the relationship between serum GFAP levels and the result of traumatic brain injury (TBI):

Table 4. Association of Serum GFAP Levels with Outcome of Head Injury

GFAP levels		Outcome				Information
		Bad		Good		
		n	%	n	%	
GFAP	High	17	62.9	10	37.1	p<0.001
	Normal	6	18.2	27	81.8	RR= 3.463

Inf: High GFAP: GFAP level ≥ 2.92 ng/ml,
Normal GFAP: GFAP level < 2.92 ng/ml

Source: data proceed

Based on the data in the table above, it can be seen that at high GFAP levels, the majority outcome is poor with a percentage level of 62.9% and a good outcome of 37.1%. Meanwhile, in normal GFAP cases, the outcome was mostly good with a percentage of 81.8% and bad at 18.2%.

Multivariate analysis can be defined as a collection of statistical models used to explore trends in multidimensional data by simultaneously examining several data variables. Multivariate data analysis extends bivariate analysis, which only considers two variables. The results of multivariate data analysis are presented in the following table:

3. Multivariate Analysis

Table 5. Multivariate analysis

	Variable	Coefficient	p	RR (IK 95%)
Step 1	High GFAP	1.460	0.08	4.305
	Negative pupil reaction	21.88	0.99	3.2
	Hypotension	21.74	0.99	2.76
	Old age	1.37	0.11	3.9
Step 2	High GFAP	1.84	0.19	6.3
	Negative pupil reaction	21.82	0.99	2.99
	Hypotension	21.48	0.99	2.13

Source: data proceed

Based on the data in table 5 above, it can be seen that in the initial step, the GFAP variable is high, it has a probability of 0.08, while in the second step, there is a difference, namely the GFAP coefficient is greater to 1.84, and the probability is 0.19. In the second variable, namely the negative pupil reaction, the coefficient is 21.88, and the probability is 0.99, while in the second step, there is a difference with the coefficient of 21.82 and the probability is still constant. In the Hypotension variable, the coefficient in step 1 is 21.74 with a probability of 0.99, while in step 2, the coefficient is smaller, showing the number 21.48 and a constant probability of 0.99.

4. Discussion

There was a significant association between serum GFAP levels and the outcome of TBI with a Relative Risk (RR) value for a poor outcome of 3,463 ($p<0.001$). So it can be predicted that someone with a GFAP level of more than or equal to 2.92 ng/ml has a 3.463 times risk of experiencing a bad outcome.

The GFAP cut-off point can predict adverse outcomes in various studies. Vos et al. (2010) got a 1.5 ng/ml value. Pelinka et al. (2004) get a cut-off point of 7.08 ng/ml. Wiesman et al., got a cut off point < 0.01 ng/dl. Thus, a separate cut-off point was determined in this study, namely 2.92 ng/ml with a sensitivity of 73.9% and specificity of 73% and an AUC of 86.2.

High GFAP levels (≥ 2.92 ng/ml) can more accurately predict adverse outcomes than other significant variables such as negative pupillary reflexes and the presence of hypotension. Glial fibrillary acidic protein is a critical component of the astrocyte cytoskeleton and is produced only by astrocytes, making it an excellent diagnostic of brain injury. However, like with other proteins, immunocytochemical (qualitative) evidence for GFAP expression has been discovered in the lens, Schwann cells, testicular Leydig cells, hepatic and pancreatic stellate cells, enteric glia, podocytes, mesangial cells, and chondrocytes. However, the existence of GFAP outside the CNS is irrelevant for research of CNS biomarkers (Petzold et al., 2010, 2014). Recent investigations have demonstrated that GFAP is detectable in serum from individuals who have sustained traumatic brain damage but is undetectable in serum from patients who have sustained multiple traumas such as fractures but do not suffer traumatic brain injury (Fraser et al., 2011; Schiff et al., 2012). The results of this study agree with several other studies examining high GFAP levels in association with poor outcomes in TBI.

Pelinka et al. (2004), prospective study (1999-2002) involving 92 subjects with severe TBI (GCS <6) with onset <12 hours. Serum GFAP levels were checked at admission and the next 10 days in the ICU. GFAP levels were compared between survivors and nonsurvivors and their association to the Glasgow outcome scale (GOS) after 3 months. In addition, the association between GFAP levels and Marshall scores, cerebral perfusion pressure (CPP), and mean arterial pressure (MAP) was also investigated. Serum GFAP levels were higher in patients with GOS 2-3 than in GOS 4-5 ($p<0.05$).

Nylen et al. (2007) investigated the association of elevated GFAP levels with poor outcomes. GFAP levels detected in 98% of CKB patients exceeded the reference GFAP levels (<0.15 mcg/l) with a median value ten times greater than the reference value. GFAP levels reach their peak 1-2 days after a TBI and return to normal within 1-2 weeks.

The study of Vos et al. (2010), a cohort observational study involving 79 TBI patients with GCS scores <12 and onset <24 hours, age >18 years, examined GFAP and S100B as biomarkers of TBI. This study validated GFAP and S100B as predictors of poor outcome with a cut off point of 1.5 ng/ml. GFAP levels increased 33.4 times in patients with poor outcomes with an RR value of 30.96. However, in this study, the outcome parameter used was different from ours; namely, the Extended Glasgow Outcome Scale (GOSE) was examined 6 months after the onset of TBI. Other parameters affecting outcome were also investigated in our study, namely pupillary reaction, hypotension, hypoxia, and ISS score, where the pupillary reaction was

associated with outcome whereas hypoxia and hypotension were not.

A contradictory study was conducted by Wiesmann et al. (2010), an observational cohort study examining the comparison of GFAP levels, S100B levels, neurological status, and CT scan findings as predictors of outcome in TBI. This study included 60 patient subjects with onset 24 hours after injury. Outcome assessment based on the Glasgow Outcome Scale was performed after 6 months. The correlation between GFAP levels at admission and outcome after 6 months was insignificant ($r=0.40$; $p>0.05$). The difference in the results of our study with those of Wiesmann et al., 2010 may be due to the time difference in outcome determination as our study examined only one month after onset. In addition, all patients with poor outcomes died after two days of onset. Several studies have also shown a decrease in GFAP levels several days after onset. Thus, our results suggest that GFAP levels predict short-term outcomes better than long-term ones. But of course, this still requires further study validation. Moreover, relatively few studies examine GFAP in TBI based on the time of its release (Schiff et al., 2012).

In this study, several factors that influence the outcome of TBI were also analyzed. There is a significant association between age, hypotension, and pupillary reactions with the outcome of TBI. The cohort study and randomized controlled trial involving 10,000 patients found that age, Glasgow coma scale, pupillary reactions, and intracranial haemorrhage affected outcomes. However, it is still not considered valid and specific enough as a diagnostic and prognostic tool in TBI patients (Vos et al., 2010).

Age is one of the demographic factors as a strong predictor of TBI mortality and outcome. Several studies have shown that older age shows worse outcomes (Lingsma et al., 2010).

After the multivariate test with logistic regression was carried out, it was found that the variables that affected the outcome from the largest to the smallest power were high GFAP levels (RR=6.3), negative pupillary reactions (RR=2.99), and hypotension (RR=2.13). This is following the research of Vos et al. (2010) where high GFAP levels were the most independent predictor of poor outcome.

Several confounding factors that play a significant role in assessing the outcome of TBI patients are the condition of sepsis during hospitalization. Of the 60 samples collected, 23 of them had poor outcomes, and all of them died in the hospital within less than 48 hours of onset; after reviewing the patient's medical record, none of the patients died from sepsis. Moreover, based on the research of Pelinka et al. (2004) found no effect of sepsis on GFAP levels during treatment.

D. CONCLUSION

The higher the serum glial fibrillary acidic protein level in the TBI patient, the worse the outcome after one month of onset. This study has validated the role of GFAP in predicting the outcome of TBI so that GFAP can be proposed as a useful biomarker and utilized in clinical practice.

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