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RESEARCH

SERUM GLIAL FIBRILLARY ACIDIC PROTEIN LEVELS PROFILE IN PATIENTS WITH SEVERE TRAUMATIC BRAIN INJURY

(Profil Kadar Glial Fibrillary Acidic Protein Serum di Pasien Cedera Otak Berat)

Arief S. Hariyanto[1], Endang Retnowati[2], Agus Turchan[3]

ABSTRAK

Glial Fibrillary Acidic Protein (GFAP) sangat khas untuk otak (highly brain specific protein), sebagai petunjuk kerusakan sel, merupakan protein yang berhubungan dengan peningkatan tekanan intrakranial dan sebagai petanda perjalanan penyakit di pasien cedera otak. Penelitian ini menganalisis profil kadar GFAP serum pasien cedera otak berat sebagai petanda perjalanan penyakit dan keluarannya. Desain penelitian deskriptif observasional. Kadar GFAP serum dari sampel darah vena, diperiksa dengan metode ELISA pada hari pertama datang ke Instalasi Gawat Darurat dan hari ke-2,3,4 perawatan. Jumlah sampel 25 orang, laki-laki 20 orang (80%), perempuan 5 orang (20%). Umur terbanyak ≤ 25 tahun, 8 orang (32%), rerata umur 35,92 ± 13,80 tahun. Jejas berdasarkan hasil CT Scan kepala terbanyak Diffuse Axonal Injury (DAI) 7 (28%), tindakan operasi sebanyak 18 (72 %), non-operasi 7 (28%), penyebab cedera, kecelakaan lalu lintas 23 (92%), jatuh 2 (8%). Rerata kadar GFAP serum hari ke-1,2,3,4 berturut-turut 2,72±1,44 ng/mL, 1,85±0,85 ng/mL, 1,67±1,26 ng/mL, 0,79±0,35 ng/mL. Keluaran pasien, hidup 19 (76%), meninggal 6 (24%). GFAP sangat khas pada otak berguna sebagai petanda di pasien cedera otak berat, yaitu peningkatan kadarnya dapat digunakan sebagai faktor perjalanan penyakit untuk kematian dan keluarannya. Peningkatan kadar GFAP serum dapat digunakan sebagai faktor perjalanan penyakit. Penelitian lanjutan diperlukan dengan sampel yang lebih besar.

Kata kunci: *Glial fibrillary acidic protein, cedera otak berat, perjalanan penyakit*

ABSTRACT

Glial Fibrillary Acidic Protein (GFAP) is a highly brain-specific protein, an indicator of cell destruction and one of the prognostic factors in brain injury. The aim of this study was to analyze serum GFAP levels profile in patients with severe traumatic brain injury as a prognostic factor and outcome. Design of this study was observational descriptive. Serum GFAP levels were examined using ELISA method on day 1 in the ER and day 2,3,4 of hospitalization. Total samples were 25 patients consisting of 20 (80%) males, 5 (20%) females. Subjects mostly were aged ≤ 25 years with a mean age 35.92 ± 13.80 years. Head CT scan revealed Diffuse Axonal Injury (DAI) 7 (28%), surgery 18 (72%), non-surgery 7 (285), causes of brain injury were traffic accidents 23 (92%), falling down 2 (8%). Serum GFAP levels on day 1,2,3,4 were 2.72 ± 1.44 ng/mL, 1.85 ± 0.85 ng/mL, 1.67 ± 1.26 ng/mL, 0.79 ± 0.35 ng/mL, respectively. Outcomes showed survived 19 (76%), died 6 (24%). GFAP is specific for the brain and as a biomarker for patients with severe traumatic brain injury, increasing levels can be used as a prognostic factor and outcome. Increased levels of serum GFAP can be used as a prognostic factor. A further research is needed with a larger sample.

Key words: Glial fibrillary acidic protein, severe traumatic brain injury, prognostic

INTRODUCTION

Brain Injuries in Indonesia are still a major cause of disability, death and require a high cost in handling. The high morbidity and mortality in patients with brain injuries at the Dr. Soetomo Hospital show that brain injury requires very comprehensive treatment, covering prehospital care and hospital care. Data from brain injury patients who came to the Dr. Soetomo Hospital since January 2002 until December 2006, showed that: The operation figures ranged from 18.87% to 25.27% of all brain injury patients who

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came to the ER; Based on the severity, mortality of patients with severe traumatic brain injury is high, between 25.13% to 37.14 %, with a tendency decreases each year. This figure is still higher than the average mortality patients in developed countries which ranged from 22%; Mortality at all severity levels of brain injuries ranged from 6.171% to 11.22%. This figure is higher than the standard in international literature, which ranges between 3-8%[6]

Glial fibrillary acidic protein (GFAP) gives strength and shape of cells on astrocytes. Glial fibrillary acidic protein found in the white and gray matter and in the upregulation of astrogliosis. Glial fibrillary acidic protein is a protein which is highly specific for the brain (highly specific brain proteins) not generated by other cells outside the central nervous system and is a highly specific marker for the brain. Current data indicate that GFAP is an indicator of cell destruction.[1,4,5,7]

Glial fibrillary acidic protein is released due to increased intracranial pressure and brain injury. Glial fibrillary acidic protein has unique abilities as both a marker for neuronal and glial cell damage in brain injury and potentially as repair predictor for damage neuron and cell death. In situations where the use of CT scans is not possible, it is necessary to have a marker of brain injury that can be used to determine the brain damage. Glial fibrillary acidic protein is just released by brain cells that undergo damage.[1-3,7,10]

Glial fibrillary acidic protein is reported as one of the important prognostic factors compared to other markers in patients with brain injury. Several studies have found that levels of serum GFAP on arrival at the hospital increased significantly in brain injury patients, the correlation between serum concentrations and types of pathological brain damage and clinical outcomes have also been reported.[4,5,7,8]

The aimed of this study was to analyze the profile of serum GFAP levels in patients with severe traumatic brain injury as a prognostic factor and outcome of brain tissue damage. The benefit of this research is an alternative prognostic factor in the hospital to monitor neuronal and glial cell damage in severe traumatic brain injury.

METHODS

This study used a longitudinal design with an observational descriptive type. In this study, serum GFAP levels were examined on day 1 brain injury and on day 2,3,4 of hospitalization. The length of study was 3 months (April–June, 2016). The study was conducted in the Emergency Room (ER) for sampling of Severe Traumatic Brain Injury patients day-1,2,3 and 4 in the Clinical Pathology Laboratory Dr. Soetomo Hospital, for examining the levels of serum GFAP.

The study population included all patients with severe traumatic brain injury who came to the Emergency Room (ER) Dr. Soetomo Hospital. Samples were patients with severe traumatic brain injury in the ER Dr. Soetomo Hospital diagnosed by a doctor based on history, clinical symptoms and Computed Tomography (CT) scan of the head. Sample acceptance criteria were more than 18 years of age, GCS 3-8 after resuscitation, abnormal head CT scan at the time-coming to the hospital less than 24 hours, while the criteria for rejection of samples was those who arrived more than 24 hours, unstable vital signs after resuscitation and pregnant females.

Venous blood samples were taken from the cubital vein about 5 mL. Venous blood was inserted into vacuum tubes without anticoagulant or serum separator tubes and allowed to clot. Centrifugation of the sample tube at 1000 G for 10 minutes to obtain serum and then inserted into the serum aliquot tube. Aliquots were then labeled and stored at a temperature -20°C in the Research and Development (R & D) Clinical Pathology Dr. Soetomo Hospital until further examination. The method used for the examination of serum GFAP levels was Enzyme Linked Immunosorbent Assay (ELISA). Glial fibrillary acidic protein levels of peripheral venous blood serum of patients measured by ELISA reader brand Biotrak II belonging to the Laboratory of Clinical Pathology, Dr. Soetomo Hospital with the unit used as ng/mL. The kit used was Human GFAP (Glial fibrillary Acidic Protein) ELISA KIT FineTest®, Wuhan Fine Biotechnology Co., Ltd.

The data was collected with data collection sheets. All data collected were subjected to coding, tabulation and data entry into the computer. Descriptive data were presented in tables, charts and graphs and numerical data as mean ± SD. This research obtained permission from the Ethics Committee of the Dr. Soetomo Hospital, Surabaya.

RESULTS AND DISCUSSION

During the period of April 2016–June 2016, 25 patients diagnosed with severe traumatic brain injury coming to the ER Dr. Soetomo Hospital were obtained. All patients underwent a CT Scan. In this study no patients who entered the exclusion criteria were found, but two patients did not meet the criteria as two patients died on arrival at the Dr. Soetomo

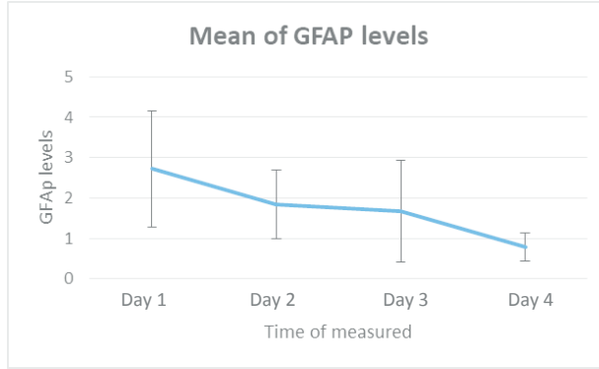

Figure 1. Mean serum levels of GFAP in severe brain injury patients

Hospital. All family research subjects of 25 patients who met the inclusion criteria and were not included in the exclusion criteria agreed to participate in the study by signing an informed consent, but there were 6 patients who died during care.

The observation made during the study was the profile of serum GFAP levels in patients with severe traumatic brain injury, in which the serum GFAP levels were observed for 4 days in a row during treatment.

Figure 1 showed the mean serum GFAP levels in patients with severe traumatic brain injury based on the day of care.

Table 1 showed the levels of serum GFAP by sex and the length of day care, showing a decrease in the mean levels of GFAP for four days in males as well as females.

Table 2 showed the mean serum GFAP levels according to age. It was found that levels vary among that age group, high levels which were not found consistently among a particular age group, possible depending on the lesion of each patient severe traumatic brain injury.

Table 3 showed the levels of serum GFAP according to diagnosis. The highest levels were found in EDH +

Table 1. Levels of serum GFAP by gender

	Levels of serum GFAP			
Gender	**Day 1**	**Day 2**	**Day 3**	**Day 4**
Male	2.78 ± 1.57	1.84 ± 0.81	1.73 ± 1.40	0.79 ± 0.32
Female	2.48 ± 0.83	1.88 ± 1.08	1.48 ± 0.75	0.80 ± 0.45

Table 2. Levels of serum GFAP by age

	Levels of serum GFAP			
Age	**Day 1**	**Day 2**	**Day 3**	**Day 4**
≤25 years	2.19 ± 0.94	1.69 ± 0.60	1.26 ± 0.58	0.80 ± 0.43
>25-35 years	3.02 ± 1.59	1.38 ± 0.13	1.18 ± 0.21	0.83 ± 0.21
>35-45 years	2.28 ± 1.26	2.18 ± 1.43	2.30 ± 2.13	0.77 ± 0.59
>45-55 years	2.60 ± 0.77	2.30 ± 0.80	2.53 ± 1.69	0.73 ± 0.15
>55 years	4.53 ± 2.46	1.60 ± 0.00	1.00 ± 0.00	0.90 ± 0.00

Table 3. Levels of serum GFAP according to diagnosis

	Mean levels of serum GFAP			
Diagnosis	**Day 1**	**Day 2**	**Day 3**	**Day 4**
DAI grade I	2.29 ± 1.07	1.54 ± 0.48	1.17 ± 0.45	0.74 ± 0.35
EDH	2.45 ± 1.33	2.00 ± 1.64	1.07 ± 0.42	0.60 ± 0.46
EDH+ICH	2.25 ± 1.78	1.95 ± 0.49	1.35 ± 0.07	1.00 ± 0.00
EDH+SDH	6.10 ± 0.00	-	-	-
ICH	3.10 ± 1.53	2.25 ± 0.90	1.65 ± 0.69	0.85 ± 0.26
SAH	1.40 ± 0.00	1.30 ± 0.00	0.80 ± 0.00	0.30 ± 0.00
SDH	2.27 ± 0.85	1.73 ± 0.68	2.90 ± 2.26	1.20 ± 0.14
SDH+ICH	3.95 ± 2.62	2.50 ± 0.00	4.90 ± 0.00	-

*DAI: Diffuse Axonal Injury, EDH: Epidural Hemorrhage, ICH: Intracerebral Hemorrhage, SDH: Subdural Hemorrhage, SAH: Sub Arachnoid Hemorrhage.

Table 4. Outcome of patients with severe traumatic brain injury

Outcome	Number	%
Alive	19	76
Dead	6	24

SDH, obtained in the patients who died on the second day, while on SAH the obtained mean serum GFAP levels were the lowest.

Table 4 indicated severe brain injury patient outcomes, with patients which were alive as many as 19 people (76%) and those who died as much as 6 people (24%). Figure 2 showed a graph of serum GFAP levels by days of treatment and patients who died had an increasing trend during treatment, while those alive showed decreased serum GFAP levels during treatment.

During the three months of data collection 25 patients with severe traumatic brain injury were obtained, with males 20 people (80%) more than females. Age group was mostly ≤ 25 years, namely 8 (32%). In 25 patients with severe traumatic brain injury, surgery was as many as 18 people (72%) and non-surgery 7 people (28%), traffic accidents as many as 23 people (92%), followed by falling down 2 people (7%). The outcome of patients who were alive as many as 19 people (72%), while as many as six people died (28%). The mean levels of serum GFAP on the first, second, third and fourth day, respectively, were 2.72 ± 1.44 ng/mL; 1.85 ± 0.85 ng/mL; 1.67 ± 1.26 ng/mL; and 0.79 ± 0.35 ng/mL.

Three patients died on the second day, one person died on the third day and two people died on the fourth day, so there was no continued sampling the next day. The mean levels of serum GFAP decreased gradually during the treatment. Three patients out of 25 patients with severe traumatic brain injury (12%) showed elevated levels of serum GFAP during treatment. Serum GFAP levels in this study decreased in 19 patients (76%) during treatment, but in two patients (8%) the levels of serum GFAP settled on the first day and the second, but decreased on the third and fourth days. Levels of serum GFAP on day hospital admission decreased significantly during treatment in patients with surgery compared with non-surgery.[4,5,7,10]

The increase in GFAP levels was very likely to occur in brain ischemia. As mentioned earlier in this study the GFAP will be secreted into the blood serum immediately after a brain injury, however, GFAP will not be secreted in patients with other trauma without brain injury. Studies at the University of Diponegoro found that blood serum GFAP levels rose ± 28 times the normal value, the value of the highest serum levels obtained at EDH volume of 100 mL with a value of GFAP levels of 1.0721 ng/mL, or 32 times the maximum normal value of 0,033 ug/L (0.033 ng/mL). Volume more than ± 20 mL will cause increased GFAP levels at around 0.2285 ng/mL or 7 times the normal value.[3]

Increased levels of GFAP showed that the brain tissue experienced severe ischemia. Ischemia was due to the addition of intracranial pressure, the addition of blood components and this caused decreased cerebral perfusion pressure. As it is known that the Cerebral Perfusion Pressure (CPP) was obtained from the Mean Arterial Pressure (MAP) minus Intracranial Pressure (ICP), CPP = MAP - ICP. The decline in brain perfusion was due to increased ICP which was not offset by an increase in MAP.[3,5,7,9]

Glial fibrillary acidic protein as a biomarker can be used to predict outcomes and risk. Parameters used included clinical characteristics (age, sex and Glasgow Coma Scale), the radiological data as compliance data. In this study, the mean decrease in serum GFAP levels during treatment occurred both in males and

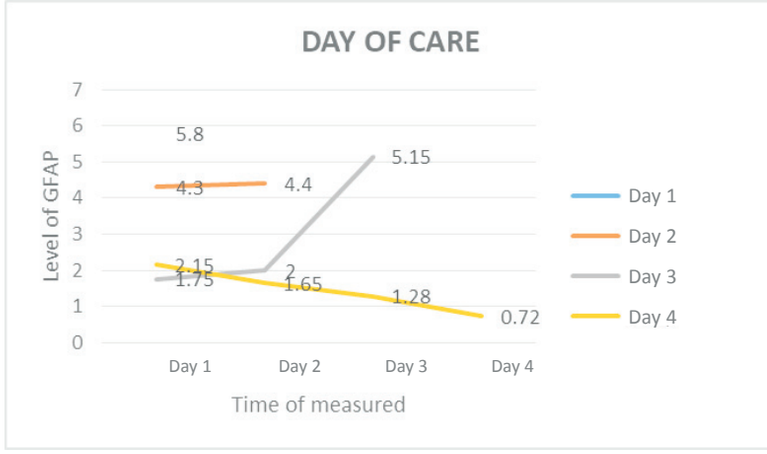

Figure 2. Levels of serum GFAP based on day of care

females. Glial fibrillary acidic protein levels showed varied serum levels among different age groups, but no high levels of a particular age group were found, the possibility of lesions depended on each patient with severe traumatic brain injury. The mean levels of highest serum GFAP were obtained in Epidural Hemorrhage (EDH) + Subdural Hemorrhage (SDH) and the patient died on the second day, while in Subarachnoid Hemorrhage (SAH) the lowest mean serum GFAP levels were obtained.

Glial fibrillary acidic protein, a specific brain protein that serves as a major component of the cytoskeleton of the astrocytes, has been reported as a biomarker in the blood. In brain damage, GFAP is released from the brain cells into the interstitial fluid and in the peripheral blood, through the blood-brain barrier damage. Some studies indicated that GFAP levels of less than 12 hours after injury were significantly among patients with improved outcomes compared to bad outcomes. In another study GFAP levels of less than 6 hours after brain injury was also associated with the outcome but were not the same when examined 24 hours after the injury. Several other studies have shown that increased levels of GFAP at 24 hours and 2 days can be used to predict mortality and poor outcomes.[3-5,7,8]

Increased levels of GFAP occurred during the first day in patients with a good outcome. Increased levels of GFAP marked in patients with poor outcomes, during the first two days and an increase in GFAP levels in patients who died during the three days, according to previous studies. Glial fibrillary acidic protein levels on the day of hospital admission could be used as a predictor for determining the outcome.[4,5,9,10]

In this study, two patients with GFAP levels were increased on the second and third days, in which the patient died on the fourth day. The two patients showed lesions in the form of SDH. The presence of subdural hematoma or epidural hematoma was associated with early increased levels of GFAP, although the initial levels of serum GFAP were not associated with mortality, levels of GFAP settled on day 2 were significantly associated with death. Increased levels of GFAP were settled on the 2nd day showed that secondary injury caused death.[3,5,8,10]

Biomarkers used in this study only examined serum. GFAP concentration in cerebrospinal fluid was higher than in the serum, but serum sample was more practical during the acute phase. In clinical practice, serum biomarkers were more possible to be examined especially in the Emergency Department. The GFAP had a half-life of < 2 days and rapidly decreased during the acute phase. Only the sample during the acute phase can be used as a predictor of outcome, especially in those who had no secondary injury.[5,7,10]

Limitations of this study, firstly, only 25 patients were sampled and only 19 patients were examined four times, six patients died were not examined four times. Secondly, this study did not analyze patients undergoing surgery. Thirdly, serum biomarkers did not fully describe the biochemical changes in the injured brain, although it an association between levels of serum GFAP with cerebrospinal fluid was reported.

CONCLUSIONS AND SUGGESTIONS

Glial Fibrillary Acidic Protein (GFAP) is specific to the brain and useful as a biomarker for patients with severe traumatic brain injury, in which an increase in levels can be used as a prognostic factor of mortality and outcome. A further research on the development of the increased level of consciousness in severe brain injury patients associated with changes in levels of blood serum GFAP with a larger sample is needed.

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