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Investigation of the course of GFAP in blood in the initial 24 hours in rats subjected to minor head trauma

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Abstract. – AIM: Aim of this study is to evaluate the diagnostic efficacy of Glial Fibrillar Acidic Protein (GFAP) particularly in minor head traumas.

MATERIALS AND METHODS: 72 female and male, 3 month-old, Sprague Dawley rats were used in the study. The rats were divided into 9 groups. Following anesthesia, all rats were placed in prone position. A 10 mm long and 3 mm thick stainless steel metal disc was fixed onto the skull using dental paste in order to sustain a closed head trauma and evenly distribute the weight throughout the skull. After placing it under the metallic pipe arrangement over a height of 80 centimeters and fixing to make it constant, 50 g metallic discs were released by free fall, and the head trauma was sustained thanks to the gravity-generated force. Blood samples were collected from the rats under anesthesia for biochemical GFAP analysis 10 minutes after the trauma and in 1, 2, 3, 4, 5, 6 and 24 consecutive hours later.

RESULTS: GFAP has a peak, and its peak level at hours 1 and 2 in rats subjected to a minor head trauma, with a slight decrease afterwards.

CONCLUSIONS: GFAP is an important marker in determining the severity of traumatic brain injury.

Key Words:
Minor head trauma, GFAP, Brain injury.

Introduction

Cranial Computed Tomography (CCT) is an important tool in the management of the diagnosis and treatment of cases with head trauma[1,2]. However, there is controversy in the literature about taking CCTs in cases with mild head trauma (MHT), which account for a great majority of cases with head trauma[3,4]. Opinions exist arguing that actions should be taken more selectively based only on clinical background or stimulating clinical environment in physical-neurological examination or no CCT scans should be done as well as opinions suggesting that CCT scans should be done in almost all cases with MHT[3-5].

The rate of CCT diagnostic imaging request by clinicians in cases with MHT varies between 5-50% in various researches[6]. And the intracranial pathology incidence in tomographies varies between 3-6% in cases with MHT[7-10]. Besides, such pathologies found in CCTs done rarely require surgical intervention[11]. In such a case, CCT scans done in result normally in about 94-97% of cases with MHT, so CCT scans are done unnecessarily for many patients. Besides, doing CCT scans come with certain risks. Doing CT scans requires sedation, which brings together many risks including hypoxia, apnea, change in level of consciousness, risk of aspiration, and even endotracheal intubation indication[12,13]. Particularly in children less than one year of age, it results in radiation exposure due to computed tomography and in associated mortality risks[14,15]. Alternative diagnostic methods are required particularly in pregnant women and infants in the diagnosis of cases with MHT.

Recently, studies are being conducted covering biomarker use in the diagnosis of Traumatic Brain Injuries[16]. One of such biomarkers is the Glial Fibrillary Acidic Protein (GFAP). The

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GFAP is a protein in the astroglial cell's skeleton and is not available outside the central nervous system. The GFAP level in the blood rises when brain or spinal cells are damaged due to trauma or disease[17]. GFAP is also related with the severity of brain injury and the outcomes of traumatic brain injury[18]. Studies have been conducted investigating the diagnostic efficacy of GFAP particularly in severe head traumas, and blood GFAP levels have been found to be associated with the severity of the trauma and mortality[19-21]. A clinical study by Zurek et al[19], which included severe cases with head trauma, found that post-TBI GFAP blood levels were higher in cases presenting with mortality, remaining higher for days, and lower in living cases, falling rapidly.

A study by Missler et al[22] shows that GFAP rapidly falls in the initial six hours following TBI. A clinical study by Wiesmann et al[23] found the blood GFAP level significantly low in cases admitted with head trauma and gave blood 6 hours later. So, GFAP was found to be insufficient in patients admitted after six hours, suggesting that its clinical use was limited. Missler et al[24] reported that GFAP could be a marker for the detection of onset neuronal damage after primary traumatic brain injury as serum level rapidly fell during the initial six hours after the trauma. GFAP rises in the initial six hours, and then the serum level rapidly falls afterwards[25]. As can be seen, differing results exist about the course of GFAP blood levels in cases with head trauma.

This study aims to investigate how GFAP (Glial fibrillary acidic protein) blood levels, an important marker of neuronal damage in rats subjected to MHT, in the initial 24 hours.

Marmarou et al [26] model of head trauma will be used with some modifications.

Materials and Methods

72 female and male, 3 month-old, Sprague Dawley rats were used in the study. The rats were divided into 9 groups. Anesthesia was given to all groups using 5 mg/kg Xylasine and 50 mg/kg Ketamine. Following anesthesia, all rats were placed in prone position. A 10 mm long and 3 mm thick stainless steel metal disc was fixed onto the skull using dental paste in order to sustain a closed head trauma and evenly distribute the weight throughout the skull. After placing it under the metallic pipe arrangement over a height of 80 centimeters and fixing to make it constant, 50-gram metallic discs were released by free fall, and the head trauma was sustained thanks to the gravity-generated force. No skull fractures and deaths occurred after the trauma. Blood samples were collected from the rats under anesthesia for biochemical GFAP analysis 10 minutes after the trauma and in 1, 2, 3, 4, 5, 6 and 24 consecutive hours later. Following the procedure, the rats were decapitated by perfusion- fixation method.

Detection of GFAP Levels

Rat GFAP levels were detected in the serum by rat GFAP ELISA kit (Eastbiopharm, Hangzhou). First, the samples and standards were injected into the well. The antibodies were labeled with enzymes and the plate was incubated for 60 minutes at 37ºC. Then, the plate was washed five times and chromogen solutions were added. It was then incubated for 10 minutes at 37ºC and stop solution was injected into the wells. Optical density (OD) was measured under 450 nm wavelengths with a microplate reader (LabSystems, UV/Vis. Spectrum Finstruments™ Multisblood Model 347, Vantaa, Finland).

Statistical Analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences for Windows, version 15.0 (SPSS Inc, Chicago, IL, USA) and Sigmastat version 3.5 (Statcon Inc., B. Schäfer, Witzenhausen GERMANY) software. Normal distribution of the data was evaluated with te Kolmogorov-Smirnov test. Friedman Repeated Measures Analysis of Variance on Ranks Test was performed to compare hours. Data were presented as median and interquartile range (25%-75% percentiles). Statistical significance was defined as $p < 0.05$.

Results

Normal distribution test was performed for blood GFAP values in rats, revealing that they were not normally distributed.

This study investigates GFAP levels in blood samples collected immediately (0 hour) post-trauma from the control group (C) and the trauma-induced group, and differences between the other groups. No statistically significant differences were, as anticipated, found between the control group and 0 hour blood GFAP levels. However, there was a noteworthy increase at hour 1 blood

Table I. GFAP values and stastical analysis.

Hours	n	Median (25%-75% percentiles)	p
Control group	8	2.176 (1.953-2.373)	
0.hour	8	2.111 (1.801-2.265)	< 0.05
1.hour	8	2.499 (2.455-2.571)	
2.hour	8	2.463 (2.035-3.067)	
3.hour	8	2.298 (2.130-2.421)	
4.hour	8	2.252 (2.208-2.294)	> 0.05
5.hour	8	2.370 (2.284-2.526)	
6.hour	8	2.401 (2.334-2.511)	
24.hour	8	2.397 (2.256-2.512)	

Difference ($p < 0.05$) was found between hours 0 and 1, with no difference between the other hours.

GFAP levels compared with hour 0. This increase was considered statistically significant ($p < 0.038$). Although blood GFAP values at 1 hour post-trauma were not considered to be statistically significant, the values were lower afterwards (3, 4, 5, 6, 24 hours) (Table I and Figure 1).

An analysis of the absorbance variable by hours showed that median blood GFAP values were very low in the control group and at the zero hour, but peaked at hour 1, with a significant tendency to decrease afterwards. An analysis of the differences revealed a statistically significant ($p < 0.038$) difference between blood GFAP values at hour 0 and hour 1, with the noteworthy decreases between hour 1 and other hours being statistically insignificant ($p > 0.05$) (Table II, and Figure 2).

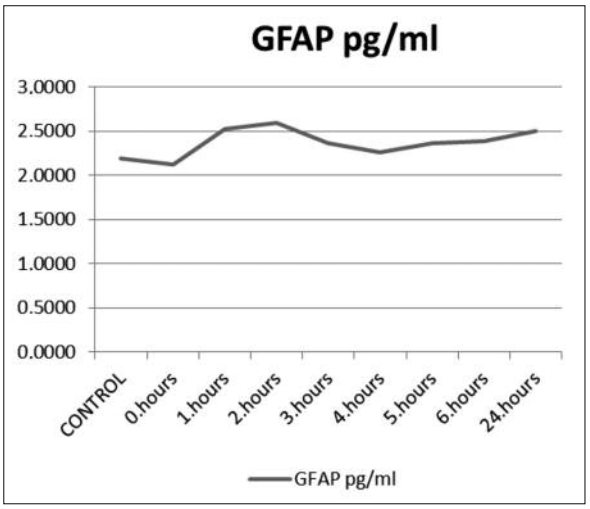

Figure 1. Hourly GFAP levels.

An analysis of the control variables (samples collected from healthy rats) by hours showed that median blood GFAP values were very low in the control group and at hour zero and peaked at hour 1, with a considerable fall afterwards. An analysis of the differences revealed a statistically significant ($p < 0.038$) difference between blood GFAP values at hour 0 and hour 1, with the noteworthy decreases between hour 1 and other hours being statistically insignificant ($p > 0.05$).

An analysis of the absorbance variable by hours showed that median Blood GFAP values were very low in the control group and at the zero hour, but peaked at hour 1, with a significant tendency to decrease afterwards. An analysis of the differences revealed a statistically significant ($p < 0.038$) difference between blood GFAP values at hour 0 and hour 1, with the noteworthy decreases between hour 1 and other hours being statistically insignificant ($p > 0.05$).

Hourly changes in GFAP values were analyzed and, as also found in the control and absorbance variables, very small changes were found in the control group and at hour zero in terms of hours, but the number of changes rapidly increased after hour 1, peaking at hour 1. There was a decrease after hour 1, with a statistically significant ($p < 0.038$) difference between hour 0 and hour 1 and the noteworthy decreases between hour 1 and other hours being statistically insignificant ($p > 0.05$).

Difference ($p < 0.05$) was found between hours 0 and 1, with no difference between the other hours. An examination of blood GFAP values showed no meaningfully high values between the control values and values at hour 0, and that

Table II. GFAP levels and absorbans values.

Hours	n	Median (25%-75% percentiles)	p
Control group	8	0.487 (0.358-0.601)	
0.hour	8	0.487 (0.358-0.601)	< 0.05
1.hour	8	0.673 (0.648-0.715)	
2.hour	8	0.652 (0.406-1.001)	
3.hour	8	0.557 (0.460-0.628)	
4.hour	8	0.531 (0.506-0.555)	
5.hour	8	0.599 (0.549-0.689)	> 0.05
6.hour	8	0.617 (0.578-0.680)	
24.hour	8	0.615 (0.533-0.681)	

Difference ($p < 0.05$) was found between hours 0 and 1, with no difference between the other hours.

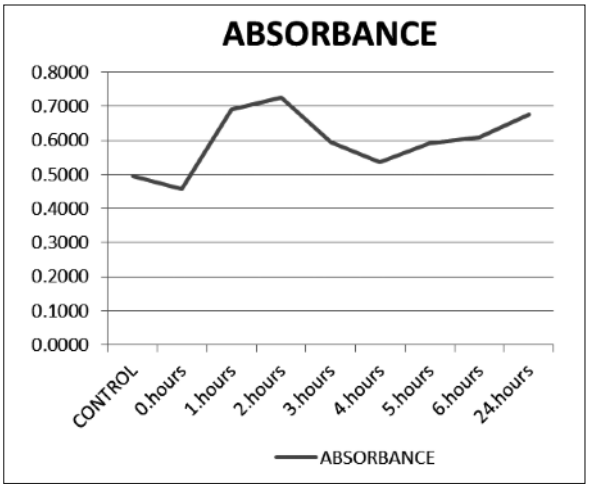

Figure 2. Absorbance variability by hours.

the rise at hour 1 was statistically meaningful ($p = 0.038$).

Discussion

CCT is an important tool in the management of the diagnosis and treatment of cases with head trauma. Today, patients who are referred to the Emergency Unit for head trauma are evaluated by clinical examination (GCS) and CCT. However, GCS has a limited effect in the evaluation of patients sedated and intubated under emergency conditions.

CCT is clearly indicated in high-risk intracranial injury situations including consciousness disorder, findings of skull base fractures, progressive neurological deficit, skull collapse fractures, open skull wounds, and penetrating head trauma[27]. Many authors[1,2] recommend CCT in case of any findings (nausea, vomiting) suggestive of traumatic brain injury, or where GCS is 13 and below. However, CCT indications are still controversial in the diagnosis of patients with MHT. Yet, the majority of patients referred to the emergency unit with head trauma are cases with MHT particularly in children[28-31]. In studies that have found the pathologic CCT finding rates to be high, the authors recommend doing CCT scans in cases with MHT with a history of amnesia or loss of consciousness[32]. Miller et al[9] suggest that a history of loss of consciousness and amnesia cannot be an indicator in severe intracranial injuries. Markers showing intracranial injury in children less than two years of age are not noticeable. Neurological anomaly, variable mental state, scalp anomalies (contusion-laceration-abrasion-cephalohematoma) and vomiting are seen as the best markers[11]. Scalp hematoma is considered as a useful indicator in showing the underlying fractures particularly in infants with MHT less than 1 years of age and radiological imaging is recommended in such patients[33]. However, some studies have demonstrated that findings including vomiting, seizures, and altered state of consciousness might not be found in cases with traumatic brain injury, so such symptoms have weak sensitivity and specificity in showing intracranial injury[1]. Wang et al[4] have reported that mild cases with head trauma in the children age group was a weak indicator of a history of loss of consciousness, and intracranial injury.

Although CCT has a high sensitivity in detecting the presence of TBI, it is unnecessary to use it in all children with minor head traumas due to its high cost, and loss of time and resources. So, the goal in children with minor head traumas is to minimize the use of unnecessary imaging methods while detecting treatable TBIs[34]. Besides, sedation is required to do CCT scans, and this brings together many risks including hypoxia, apnea, change in level of consciousness, risk of aspiration and even endotracheal intubation indication[12,13]. Exposure to radiation due to CCT particularly in children less than one year of age results in malignities and associated risk of mortality[14,15].

The rate of CCT diagnostic imaging request by clinicians in cases with MHT varies between 5-50% in various reports[6]. And the intracranial pathology incidence in tomographies varies between 3-6% in cases with MHT[7-10]. Besides, such pathologies found in CCTs done rarely require surgical intervention[11]. In which case, CCT scans done in about 94-97% of cases with MHT are normal, with many patients receiving unnecessary CCT scans. Therefore, alternative diagnostic methods are required particularly in pregnant women and infants in the diagnosis of cases with MHT.

Recently, researches are being conducted covering biomarker use in the diagnosis of traumatic brain injuries (TBI)[16]. GFAP is one of such biomarkers. GFAP does not rise in the blood in patients with multiple traumas having no TBI. So, GFAP is a brain-specific marker unlike S100B, which is affected from hemorrhagic shock and extracranial trauma[21].

Studies have been conducted investigating the diagnostic efficacy of GFAP particularly in severe head traumas, and blood GFAP levels have been found to be associated with the severity of

the trauma and mortality[19-21]. A clinical study by Zurek et al[19], which included severe cases with head trauma, found that post-TBI GFAP blood levels were higher in cases presenting with mortality, remaining higher for days, and lower in living cases, falling rapidly. The same work found blood GFAP levels to be meaningfully higher in the group with TBI injury compared with the group with no TBI.

In a study by Lumpkins et al[20] where they investigated adults with severe head traumas, blood GFAP levels were found to decrease significantly on the second day. In the same study, blood GFAP levels were found to be meaningfully higher in the group with TBI compared with the control group. Again, in the same report, GFAP levels were found to be significantly higher in cases who received surgical treatment compared with cases who received no surgical treatment. In cases receiving craniectomy or craniotomy, blood GFAP levels were found to be 9.2 pg/ml and 1.9 pg/ml on day 1 and 2, respectively, and blood GFAP levels were found to be 1.1 pg/ml and 0.5 pg/ml on day 1 and 2, respectively, in persons receiving no surgical intervention. Lumpkins et al[20], found that onset GFAP levels were not associated with mortality, and that high GFAP levels maintained on the second day were meaningfully correlated with mortality.

Missler et al[21], in their study, investigated the blood GFAP level in patients with severe head traumas with a GCS ≤6. This study found the GFAP to be high in all of blood samples collected within three hours after injury, and in 56% of blood samples collected between 4-6 hours, and only 10% of blood samples collected after 7 hours. Missler et al[22] found that GFAP has rapidly fallen in the initial six hours following TBI. And, according to Pelinka et al[21] the level remained high for days in cases without severe head trauma in the non-living, and fell in the initial 36 hours in the living. The same paper suggests that GFAP is a good indicator of post-TBI mortality and one of the strongest diagnostic markers in the early post-TBI period.

Our investigations show that the number of studies focusing on the diagnostic efficacy of GFAP on MHTs is limited. On the other hand, studies show that MHTs account for an important portion of childhood injuries[35,36]. Cases with a GCS of 14-15 are considered to have mild, cases with a GCS of 13-9 are considered to have moderate, and cases with a GCS of 8 and lower are considered to have severe head traumas[37].

A clinical work conducted by Wiesmann et al[23] included 60 cases with GCSs ranging between 3-15. The study found the blood GFAP levels to be meaningfully high in the initial 6 hours, and significantly low in blood samples collected after 6 hours. As a result, GFAP was considered to be a good marker for neurological results in cases who admitted and gave blood in the initial six hours following head trauma. However, GFAP was found to be insufficient in patients admitted after the sixth hour. So, its clinical use was found to be limited. Missler et al[24], showed that GFAP serum level increased in 12 of the 25 patients with head traumas. GFAP can be a marker for the determination of onset neuronal damage after primary traumatic brain injury as the serum level rapidly decreases in the initial six hours after the trauma.

GFAP is considered to be a promising marker in the detection of primary brain injury. GFAP rises in the initial six hours, then the serum level is rapidly reduced thereafter. Further studies are needed in order to be able to use it as a marker for the detection of primary brain damage and see its results in the pediatric population[25].

We, in this paper, investigated the course of GFAP blood levels in the initial 24 hours in rats subjected to MHT. An examination of the results revealed that blood GFAP level rose in the initial 1 hour, which was found to be statistically significant ($p = 0.038$). While blood GFAP level peaked after 1 and 2 hours, it slightly fell thereafter. However, this fall was not found to be statistically significant. And, this finding is consistent with previous studies.

The rise of GFAP blood levels in the initial hours and its fall after the sixth hour in cases with MHT is considered to be a disadvantage, and this limits the diagnostic efficacy of GFAP. However, many previous investigations report that a majority of patient with head traumas (73-93%) admit to hospitaly in the initial six hours[29,30,38,39]. Therefore, GFAP in the blood can be used as a significant marker in a great majority of the cases with MHT.

Intracranial pressure increase and fall in cerebral perfusion pressure, which are results of primary traumas in severe head traumas, result in secondary damage and cellular damage. This can explain why, while many studies[20,22,23] found the GFAP blood level to decrease in the early period, blood GFAP level remained high for days in studies which included cases with severe head traumas[21].

Childhood head traumas are still one of the most significant public health concerns of the world and is one of the most common causes of morbidity and mortality in this age group[39-41]. Studies show that in the United States of America (USA) more than 500,000 children are admitted to the emergency units for head traumas, 60,000 are treated in hospitals, and 7,000 are lost[43,44].

The algorithm to be followed in cases with childhood MHT poses difficulties both for the physician and the family. The physician, while trying to make the accurate diagnosis for the patient, chooses to do tomography scans, although not necessary at times, due to both ethical and legal obligations (the law of malpractice and associated mandatory professional insurance), struggle not to make any wrong diagnoses. We tried to find an answer to the question of "Can GFAP, an indicator of neuronal damages in head trauma, be allowed to be clinically used?" just like TROPONIN-T, a marker used in the diagnosis of myocardial infarct. This will allow diagnosing the patients with blood tests doing no tomography scans, which is more economical compared with CCT in cases with MHT, and avoid risks including radiation.

Conclusions

We consider that GFAP, an important marker in determining the severity of TBI and mortality in severe head traumas, could also be used as a diagnostic marker in cases with MHT. There are a limited number of studies investigating the efficacy of GFAP in diagnosing MHT. Further studies are needed for GFAP to be used as an alternative diagnostic method for CCT in mild cases with head trauma.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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