RESEARCH PAPER

Acta Neurobiol Exp 2024, 84: 80–88
DOI: 10.55782/ane-2024-2543

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Evaluation of neurogranin levels in a rat model of diffuse axonal injury

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Diffuse axonal injury (DAI), one of the most common and devastating type of traumatic brain injury, is the result of the shear force on axons due to severe rotational acceleration and deceleration. Neurogranin (NRGN) is a postsynaptic protein secreted by excitatory neurons, and synaptic dysfunction can alter extracellular NRGN levels. In this study, we examined NRGN levels in serum and cerebrospinal fluid (CSF) after experimental DAI in terms of their diagnostic value. Experimental DAI was induced using the Marmarou technique in male Wistar albino rats. Serum and CSF NRGN levels of the sham group, one-hour, six-hour, 24-hour, and 72-hour post-DAI groups were measured by ELISA method. DAI was verified by staining with hematoxylin-eosin and β-amyloid precursor protein in the rat brain samples. While no histopathological and immunohistochemical changes were observed in the early hours of the post-DAI groups, the staining of the β-APP visibly increased over time, with positivity being most frequent and intense in the 72-hour group. It was found that serum NRGN levels were significantly lower in the 6-hour group than in the sham group. The serum NRGN levels in the 24-hour group were significantly higher than those in the sham group. This study showed a dichotomy of post-DAI serum NRGN levels in consecutive time periods. NRGN levels in CSF were higher in the one-hour group than in the sham group and returned to baseline by 72 hours, although not significantly. Our study provides an impression of serum and CSF NRGN levels in a rat DAI model in consecutive time periods. Further studies are needed to understand the diagnostic value of NRGN.

Key words: axonal damage, diffuse axonal injury, neurogranin, rat model, traumatic brain injury

INTRODUCTION

Diffuse axonal injury (DAI) is one of the most common and devastating types of traumatic brain injury (TBI) (Iwata et al., 2004). It is the result of the shear forces on the axons that occur durring severe rotational acceleration and deceleration of the brain within the skull. It is characterized by widespread white matter damage and is the main cause of impaired consciousness after severe head trauma (Christman et al., 1994; Li and Feng, 2009). Pathologically, DAI is associated with a primary mechanical breakdown of the axonal cytoskeleton and a range of effects from skeletal disruption to secondary physiological changes, axonal swelling, lobulation and proteolysis (Gultekin and Smith, 1994). Microtubule damage leads to impaired axonal transport of molecules (Gennarelli, 1993; 1996). Calcium both mechanically accumulates in the cell and hydrolyzes calpastatin by disrupting calcium channels and mitochondria. Calpain is activated and hydrolyzes the cytoskeleton and microtubules (Bruggeman et al., 2020). This cascade leads to apoptosis and disconnection of the axon and concomitant loss of synapses and reduction in synaptic protein expression (Canty et al., 2013; Jamjoom et al., 2021).

Received 4 December 2022, accepted 5 December 2023

Neurogranin (NRGN) is a postsynaptic protein concentrated on dendritic spines that links calcium/calmodulin and protein kinase C (PKC) signaling in synaptic plasticity (Repress et al., 1990; Guadaño-Ferraz et al., 2005). It is a substrate of PKC, a brain-specific, calcium-activated, phospholipid-dependent kinase (Repress et al., 1990). Non-phosphorylated NRGN binds to calmodulin to regulate calcium flux in the absence of calcium. NRGN is believed to easily pass a damaged the blood-brain barrier due to its relatively small size of 7.5 kD (Díez-Guerra, 2010). In pathologies such as mild TBI and Alzheimer's disease, NRGN levels in serum and cerebrospinal fluid (CSF) may fluctuate due to synaptic dysfunction (Kaleka & Gerges, 2016; Peacock et al., 2017; Portelius et al., 2015; Svirsky et al., 2020; Yang et al., 2015). Therefore, NRGN has the potential to be used for early diagnosis of such pathologies.

Diffuse axonal injury is known to increase the long-term risk of neurodegeneration and dementia. Early diagnosis and the treatment of reversible brain damage is therefore essential to optimize both short- and long-term outcomes (Graham et al., 2020). Sensitive and specific diagnostic methods have been investigated to enable early treatment (Gennarelli et al., 1982; Li et al., 2009; Mu et al., 2019; Wasserman et al., 2016). In this study, we hypothesized that extracellular NRGN levels are impaired after axonal injury associated with synaptic disruption. Therefore, we aimed to examine serum and CSF NRGN levels in a rat DAI model in consecutive time periods to determine the likely potential of NRGN as a diagnostic biomarker for DAI.

METHODS

This study was approved by the Ethics Committee of Yeditepe University (Istanbul, Turkey) (Approval date and number: October 22, 2019; 2019/10). Animal experiments were performed in accordance with the guidelines of all relevant animal welfare laws of the European Union.

Forty adult male Wistar albino rats (12-16 weeks old, weighing 350-400 g) were obtained from Yeditepe University Faculty of Medicine Experimental Research Centre (Istanbul, Turkey). Rats were housed in groups of four animals per cage at room temperature of 22°C to 25°C and humidity of 60-80% with a 12 hours/12 hours light/dark cycle. Food and water were available *ad libitum*.

Animals and trauma model

Rats were divided into five groups of eight animals each, including a sham group, a one-hour, a six-hour, a 24-hour, and a 72-hour post-DAI group. All groups except the sham group were exposed to head trauma.

The Marmarou closed head trauma weight-dropping model was used to create diffuse axonal brain injury in the rats (Foda and Marmarou, 1994; Marmarou et al., 1994; 2009). All components of the model (weight of the trauma device, drop height, and thickness and stiffness of the foam bed) were designed to represent the upper limit of severe injury. A weight of 500 g was dropped in free fall from a height of 210 cm. A general anesthetic consisting of 50 mg/kg ketamine hydrochloride and 10 mg/kg xylazine was administered intraperitoneally to each rat. The four experimental DAI groups were sacrificed one, six, 24, and 72 hours after DAI and the sham group 72 hours after anesthesia.

Measurement of NRGR levels

Venous blood from the jugular vein and CSF samples from the cisterna magna (by the cisternal puncture method) were obtained from all post-DAI groups before they were sacrificed. Blood samples were taken from the sham group for statistical comparison one, six, 24, and 72 hours post-DAI groups. CSF samples were also taken from the sham group 72 hours before they were sacrificed.

Blood samples were centrifuged at 4000 rpm for 10 minutes. CSF was poured directly into an Eppendorf tube and placed in a -80°C oven. A rat NRGN enzyme-linked immunosorbent assay kit (catalog number: E2458Ra, Bioassay Technology Laboratory, Shanghai, China) was used to measure NRGN levels. The standard curve range of this kit was between 1 ng/mL and 400 ng/mL with a sensitivity of 0.6 ng/mL. This was used for accurate quantitative determination of rat NRGN level. The kit plate was pre-coated with rat NRGN antibody. Each rat sample was placed in a well of the plate, and the NRGN in the sample was bound to the antibody coated on the wells. The rat biotinylated NRGN antibody was then added, which also bound to the NRGN in the sample. Streptavidin-horseradish peroxidase was added, which bound to the biotinylated NRGN antibody. After incubation, the unbound streptavidin-horseradish peroxidase was washed off. A substrate solution was then added and the sample turned yellow in proportion to the amount of rat NRGN in the sample. The reaction was stopped by adding an acidic stop solution and the absorbance was measured at 450 nm.

The optical density value of the serum and CSF NRGN levels were determined and recorded with a DAR800 microplate reader (Diagnostic Automation

Inc., Calabasas, CA, USA). Calculations were performed using curve-fitting software (Kcjunior for Windows, version 1.4, Bio-Tek, Vermont, USA) and the best fit line was determined by regression analysis. The resulting NRGN standard curves were recorded.

Pathological and immunohistochemical examination

After sacrification, each rat was decapitated, taking care not to damage the brain or brainstem, and histopathological and immunohistochemical examinations were performed. Brain and brainstem samples were collected from each rat, fixed with 10% formaldehyde solution, and stored for 1–2 days. Subsequently, a minimum of five coronal cryostat brain sections were obtained, with the regions of interest focused on the hippocampal areas, corpus callosum, and rostral brain stem (from a unit at +2.0 to -6.0 mm relative to the bregma). Specimens were embedded in paraffin blocks and serial sections of 5 µm thickness were taken and stained with hematoxylin and eosin (H&E).

Beta-amyloid precursor protein (β-APP) 228 antibody (catalog number: MO20015, Neuromics, MN, USA) was used for immunohistochemical analysis. For immunohistochemical fixation, the paraffin sectioned slides were preheated to 72°C in an oven device. They were incubated with the APP antibody for 1 hour and 40 min. After deparaffinization, 3 µm thick sections were collected on glass slides. The slide sections were heated in an oven at 60°C for 20 minutes. The antibody was titrated at a dilution of 1:50. Cytoplasmic brown staining of axons by the β-APP primary antibody was considered positive for the presence of DAI. The preparations were examined by a pathologist under a light microscope (Zeiss, Oberkochen, Germany) at ×40 and ×100 magnifications.

Table 1. Comparison of serum neurogranin levels.

Groups	Mean ± STD (ng/mL)	*P*
Sham group	51.42 ± 7.45	
One-hour post-DAI group	49.03 ± 9.40	
Six-hour post-DAI group	38.75 ± 8.24	0.003*
24-hour post-DAI group	65.45 ± 14.13	0.026*
72-hour post-DAI group	53.68 ± 7.91	

*ANOVA test (*P*<0.05 statistically significant) DAI: Diffuse axonal injury / STD: Standard deviation.

Statistical evaluation

Data were analyzed with SPSS version 25.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were presented as mean ± standard deviation or median and range for continuous variables. Conformity of the data to a normal distribution was assessed with a Shapiro-Wilks test, and homogeneity of variances was evaluated with a Levene test. The ANOVA was used to compare normally distributed CSF and serum values with homogeneous variance between groups. The Kruskal-Wallis test was used to compare non-normally distributed data between groups. When a statistically significant difference was found between the mean NRGN values of the different groups, the Tukey test was used to determine between which groups the difference existed. *P* value < 0.05 was considered statistically significant.

RESULTS

Serum neurogranin analysis

The mean serum NRGN levels of the trauma groups compared with those of the sham group are shown in Table 1. Among the experimental groups, the serum NRGN levels of the six-hour group were significantly lower than those of the sham group ($F4,55=4.279$, $P<0.001$), and the serum NRGN levels of the 24-hour group were significantly higher than those of the sham group ($t34=-1.977$, $P=0.026$, $P<0.05$). A dichotomy was observed in the results. There were no significant differences between the NRGN levels of the sham group and those of the one-hour and 72-hour groups (Fig. 1).

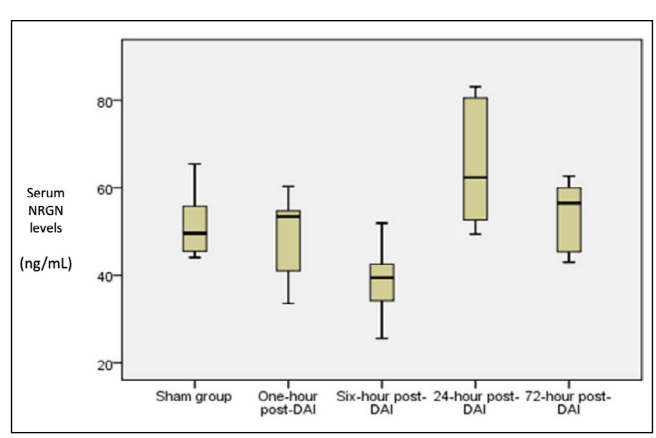

Fig. 1. Boxplot of serum neurogranin levels. Structures: (DAI) diffuse axonal injury; (NRGN) neurogranin.

To determine the differences between the groups, the Tukey test was performed (Table 2). It showed that the serum NRGN level in the 24-hour group was 1.34 times higher than in the one-hour group and 1.69 times higher than in the six-hour group. These differences were statistically significant (P<0.05).

Table 2. Comparison of the differences between serum neurogranin levels of DAI groups.

Experimental groups		Mean Difference	(95% CI)	*P*
One-hour post-DAI	Six-hour post-DAI	10.28	(-3.68, 24.25)	0.209
	24-hour post-DAI	-16.42	(-30.38, -2.45)	0.017*
	72-hour post-DAI	-4.65	(-18.61, 9.31)	0.800
Six-hour post-DAI	24-hour post-DAI	-26.70	(-40.67, -12.73)	0.000*
	72-hour post-DAI	-14.93	(-28.90, -0.96)	0.033*
24-hour post-DAI	72-hour post-DAI	11.77	(-2.19, 25.73)	0.122

*Tukey honestly significant difference (P<0.05 statistically significant) CI: Confidence interval / DAI: Diffuse axonal injury.

CSF neurogranin analysis

The comparison of the mean CSF NRGN levels of the trauma groups and the sham group is shown in Table 3 (KW4=8.058, P=0.089, P>0.05). Compared with the CSF NRGN values of the sham group, the values of the one-hour group were increased 1.61-fold, those of the six-hour group 1.4-fold, those of the 24-hour group 1.3-fold, and those of the 72-hour group 1.25-fold. However, none of these differences were statistically significant. Among the DAI trauma groups, it was observed that the CSF NRGN levels were highest in the one-hour group; and then gradually decreased with each subsequent time group (Fig. 2).

Histopathological findings

Microscopic examination of H&E-stained sections revealed a 30% rate of DAI (9/30 sections). Morphologically, DAI was not detected with H&E in the one-hour and six-hour groups. The nine sections with histopathological changes belonged to the 24- and 72-hour groups. Of these, six sections were from the 24-hour group (75%) and three were from the 72-hour group (37%). In the 24-hour group, decreased neuron counts, focal edema, axonal swelling, and oval axon bulbs of 5–40 µm were observed (Fig. 3A). In the 72-hour group, there was a lesser decrease in neuron counts and occasional focal neuronal apoptosis. Fewer axon bulbs were

Table 3. Comparison of CSF neurogranin levels.

Groups	Mean ± STD (ng/mL)	*P*
Sham group	69.06 ± 6.34	
One-hour post-DAI group	111.12 ± 17.90	0.089
Six-hour post-DAI group	96.72 ± 33.21	
24-hour post-DAI group	77.05 ± 21.46	
72-hour post-DAI group	86.01 ± 9.49	

*Kruskal-Wallis test (P<0.05 statistically significant) DAI: Diffuse axonal injury / STD: Standard deviation.

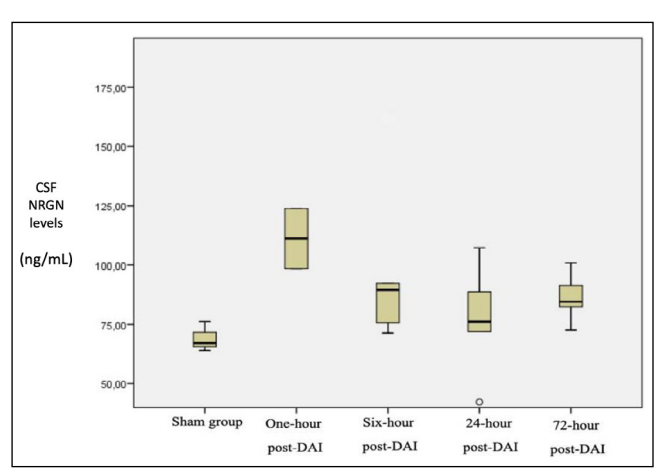

Fig. 2. Boxplot of CSF neurogranin levels. Structures: (CSF) cerebrospinal fluid; (DAI) diffuse axonal injury; (NRGN) neurogranin.

seen. Microglial nodules and areas of gliosis were also seen (Fig. 3B).

Immunohistochemical results

β-amyloid precursor protein evaluation revealed DAI in 14 of the 30 rats in the DAI group (47%). In all sections in which axonal damage was detected by H&E, axonal damage was also seen by β-APP. In the remaining five, this damage was only evident with β-APP. No morphological findings were noted in the one-hour group. Of the 14 sections in which DAI was detected, two were from the six-hour group (25%), five from the 24-hour group (62%), and seven from the 72-hour group (87%). Partial decrease in neuron number, areas of focal edema, and neuronal apoptosis were again detected in samples from the six-hour, 24-hour, and 72-hour groups.

The staining of the β-APP visibly increased with time. The β-APP positivity was most frequent and intense in the 72-hour group (Fig. 4A). In this group, diffuse and intense positive axon bulbs in the rostral brainstem and a zigzag staining pattern were observed (Fig. 4B).

DISCUSSION

Diffuse axonal injury is the most common cause of posttraumatic consciousness impairment. Clinical manifestations may vary depending on the degree of axonal damage and the location of the lesions (Li et al., 2009; Wasserman et al., 2016). As a result, histopathological and radiological classification systems; have been developed and are divided into three categories (Adams et al., 1989; 1999). Mild/lobar type DAIs are confined to the lobar white matter and are charac-

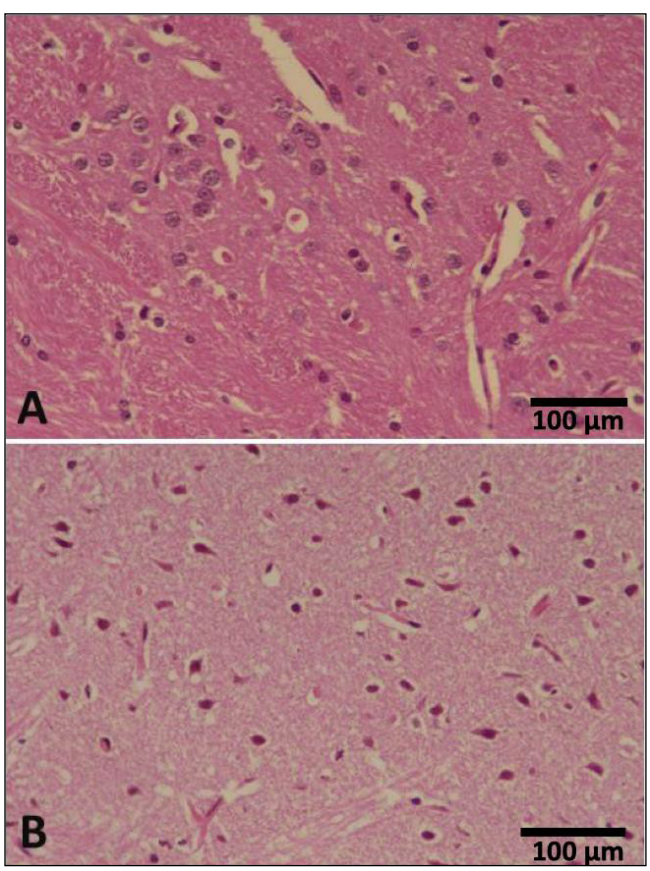

Fig. 3. Coronal section through the corpus callosum and the hippocampal areas with H&E staining. (A) decreased neuron counts, focal edema, axonal swelling, and oval-shaped axon bulbs of 5–40 µm in the 24-hour post-DAI group compared with sham and; (B) fewer axon bulbs but apparent microglial nodules and areas of gliosis in the 72-hour post-DAI group. Structures: (DAI) diffuse axonal injury; (H&E) hematoxylin and eosin.

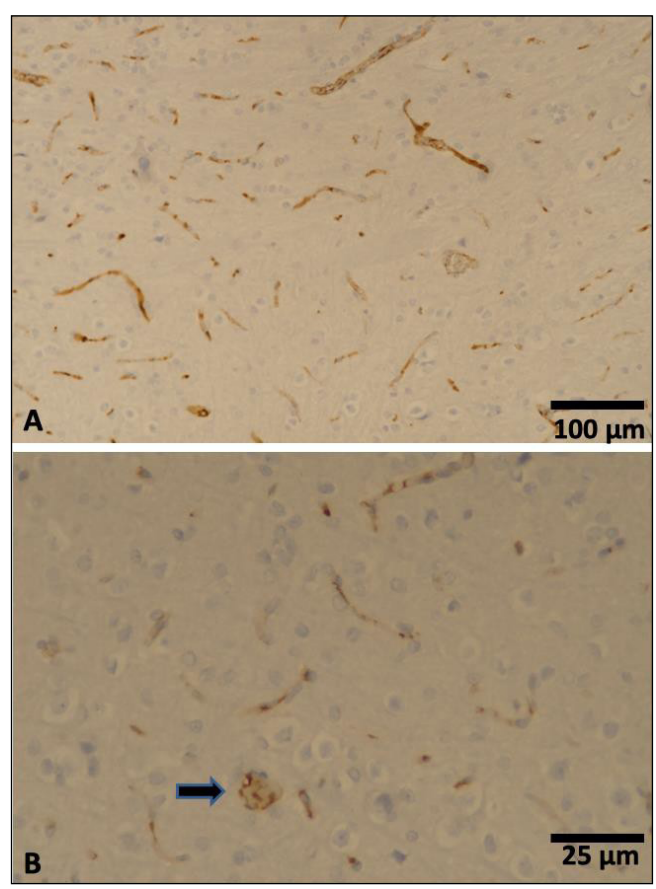

Fig. 4. Coronal section through the rostral brain stem β-APP staining. (A) zigzag wavy staining pattern in the 72-hour post-DAI group; (B) diffuse and intensely positive axon bulbs in the 72-hour post-DAI group (arrow indicates axon bulbs). Structures: (β-APP) beta-amyloid precursor protein; (DAI) diffuse axonal injury.

terized by axonal bulbs or axonal swellings. In moderate/callosal type DAIs, the corpus callosum is affected and hemorrhagic and necrotic lesions occur. In the severe/brainstem DAI type, the brainstem is affected (Adams et al., 1989; Haber et al., 2017). According to this classification, 14%, 43%, and 43% of the subjects in our study were mild/lobar type, moderate/callosal type, and severe/brainstem type, respectively.

Brain specimens from the rat groups were examined histopathologically and immunohistochemically to diagnose DAI and evaluate the accuracy of our trauma model. Axonal damage was examined with H&E staining. However, H&E has low specificity and sensitivity in detecting DAI. Axonal swelling and morphological changes such as axon bulbs can be detected at the earliest 12 hours after brain trauma (Foda et al., 1994; Marmarou et al., 1994; Sherriff et al., 1994b; Wilkinson et al., 1999). Therefore, morphological evidence of DAI could not be detected by H&E in our groups one and six hours after DAI. Moreover, morphological evidence of DAI was found in only nine of the 30 (30%) rats in the experimental group using this method.

In stretched and damaged axons, there is an increase in β-APP, which behaves like a microglial acute phase reactant (Johnson et al., 2013). Therefore, β-APP labels only damaged axons in assays (Gorrie et al., 2002). It is; therefore; considered the most sensitive and specific marker for early histopathological diagnosis of DAI (Sherriff et al., 1994b, 1994a; McKenzie et al., 1996). β-APP evaluations for DAI can detect axon bulbs, which are accepted pathognomonic indicators of DAI, but only at the earliest four or five hours after trauma (Iino et al., 2003). Studies have shown that β-APP levels continue to increase for some time after head trauma, peaking on the third day and then gradually returning to baseline levels by the tenth day (Geddes et al., 2001; 1997; Lewén et al., 1995; Sherriff et al., 1994a). In our study, evidence of DAI was found in 14/30 of the β-APP-stained samples (47%). While in the six-hour post-DAI group only two cases were positive, more staining was observed in the 24- and 72-hour post-DAI groups.

Serum biomarkers are frequently used in the diagnosis of a variety of pathologies, including those resulting from trauma. They can be an easy, fast, and cost-effective means of determining the prognosis and sequelae of a patient's condition. There is a great deal of research interest in using the S100 beta protein, a calcium-binding protein as a biomarker for moderate TBI (Shakeri et al., 2013). There are also numerous ongoing studies on other diagnostic markers for TBI, including ubiquitin C-terminal hydrolase-L1, glial fibrillary acidic protein, amyloid protein, neuron-specific enolase, tau protein, and myelin basic protein, but most of them lack sufficient clinical data (Berger, 2006: Cikriklar et al., 2016: Simpson et al., 2017; Wang et al., 2018). The specificity and sensitivity of potential TBI biomarkers may vary depending on whether the marker occurs in other body regions outside the central nervous system. Although such biomarkers are invaluable, the available options for diagnosing TBI may not be sensitive enough because not all TBIs cause diffuse damage and the consequences of different types may vary. Therefore, different biomarkers are needed to indicate and diagnose the presence of axonal damage and DAI.

Biomarkers that have been proposed for the diagnosis of DAI include neurofilaments, tau proteins, spectrin degradation products, and amyloid-beta peptides (Tsitsopoulos et al., 2017). Serum and CSF levels of all of these substances have been reported to increase following axonal damage, and this is particularly evident for light chain neurofilaments. The level of increase has also been found to correlate with the extent of the clinical manifestations and the severity of the damage (Žurek et al., 2011; Gatson et al., 2014). Four different types of tau protein have been studied because it is a structural axonal protein and a correlation between CSF and serum tau protein levels and axonal damage has been demonstrated (Zemlan et al., 1999; Öst et al., 2006). Studies of spectrin degradation products in DAI rat models have shown that they can be detected minutes after the onset of DAI (Huh et al., 2006; Thompson et al., 2006). In light of these promising results, research into the pathophysiology of DAI and the search for specific cellular biomarkers of axonal damage and its concomitant synaptic effects will continue.

It is well known that secondary axotomy plays an important role in the pathogenesis of axonal damage after DAI (Adams et al., 1999; Pittella et al., 2004). Processes resulting from secondary axotomy include an increase in intracellular calcium due to damaged calcium transport mechanisms that trigger activation of PKC (Wellington et al., 2016; Casaletto et al., 2017; Blennow and Zetterberg, 2018; Headley et al., 2018). It also stimulates the calpain and calcineurin cascade, impairs axonal conduction and activates cellular apoptosis (Finnie, 2016). Mechanical disruption of the axon results in accumulation of APP, results in either varicosities along the length of the axon or, in the case of complete disruption, an axonal bulb (Johnson et al., 2013). Experimental models examining the synapse after axonal injury revealed that axonal damage concurrently affects synapses located proximal and distal to the injury site (Canty et al., 2013). Nevertheless DAI also triggers several molecular and cellular

mechanisms that affect synapse maintenance, number, function, and production (Jamjoom et al., 2021).

Neurogranin is a neuron-specific postsynaptic protein secreted by excitatory neurons and is a PKC substrate involved in synaptic plasticity and cognitive functions (Repress et al., 1990; Guadaño-Ferraz et al., 2005). NRGN can easily pass the damaged the blood-brain barrier due to its relatively small size (Díez-Guerra 2010). It is expected that NRGN will be useful for detection of brain injury. Following cell injury, intracellular NRGN levels decrease (Li et al., 2008; Koob et al., 2014; Kvartsberg et al., 2015). Extracellular NRGN levels increase first in CSF and then in serum (Portelius et al., 2018; Liu et al., 2020).

NRGN has been previously studied as a potential biomarker of non-traumatic intracranial diseases. Wellington et al. (2016) reported an increase in the level of CSF NRGN in neurodegenerative diseases and high levels of CSF NRGN were found to be associated with disease of cognition, memory, and executive functions. Therefore, NRGN has been proposed as a diagnostic biomarker for Alzheimer's disease (Remnestål et al., 2016).

The relationship between TBI and NRGN levels was first investigated by Yang et al. (2015), who found that serum NRGN levels were significantly higher in TBI patients. However, in their study, the severity of TBI was not classified, and serum samples were collected from patients at a single time point (within the first 24 hours after injury). Peacock et al. (2017) found that serum NRGN levels of patients with mild TBI were significantly higher than those of a control group within 2-6 hours of injury. In their study, in which an initial blood sample was collected only in the first 24 hours after injury, a fluctuating trend was observed. This significant difference in NRGN levels disappeared in time ranges of 6-12 hours and an upward trend was observed toward 24 hours. In a recent study examining serum levels of NRGN, glial fibrillary acidic protein, and S100 beta protein in patients with mild TBI, NRGN levels were found to be significantly higher in patients with traumatic intracranial lesions than in patients without intracranial traumatic pathology (Çevik et al., 2019). In this study, too, a blood sample was only taken in the first 4 hours after the injury. Another study reported decreased NRGN levels in the brain parenchyma after TBI (Svirsky et al., 2020).

These different results appear to be due to an initial increase in serum NRGN levels resulting from cellular damage during the acute period after trauma, followed by a decrease in levels due to leakage arising from subsequent the blood-brain barrier dysfunction (Yang et al., 2015; Peacock et al., 2017). The dynamics between DAI and NRGN have not yet been studied, but may be considered similar in pathophysiology to the relationship between TBI and NRGN. Although it can be concluded from these previous studies that similar changes in NRGN levels occur in DAI, the present study is the first to investigate this experimentally. This is also the first study to examine serum and CSF NRGN levels at consecutive time intervals after DAI in a rat model.

This study has several limitations. Since it was a preliminary study, we planned the number of rats at the lowest level. Although β-APP is considered the most sensitive marker for histopathological diagnosis of DAI, its sensitivity might be influenced by postinjury survival time and microglial cell activation toward axonal injury (Lambri et al., 2001). Although DAI could not be detected histopathologically in all rats, we had accepted all traumatised rats as the DAI group because the Marmarou closed head trauma with weight dropping model is used to replicate DAI. Some other limitations have been encountered in obtaining CSF from rats. The CSF volume in rat brain is about 90 µL, and the CSF production rate ranges from 2.66 to 2.84 µL/min (Chiu et al., 2012). Some of the CSF samples were found to be traumatized and contaminated with blood by the cisternal puncture method, or CSF samples could not be obtained from some rats, or a small amount of CSF could not be included in the analysis. Therefore, CSF NRGN values could not provide homogeneity. Despite these limitations, our study provides an impression of serum and CSF NRGN levels in a rat DAI model in consecutive time periods.

The CSF NRGN levels of all DAI groups in our study were higher than those of the sham group, but not significantly so. Nevertheless, the greatest difference was seen in the group one-hour post-DAI, with levels gradually decreasing at each subsequent time point. This early detection of increased CSF NRGN, one-hour after axonal injury, appears to have the potential to detect axonal damage early. However, the need to prioritize hospitalization and stabilization of trauma patients and the difficulty of obtaining CSF so soon after injury are obstacles to this form of early diagnostic investigation.

Unlike CSF, serum can be easily obtained from blood samples. And it was assumed that levels would increase consistently and significantly after DAI NRGN, as with a TBI. However, a dichotomy was observed in the results. While serum NRGN levels were significantly lower after 6 hours, serum NRGN levels were significantly higher after 24 hours. Moreover, the changes in NRGN levels at different time points after DAI may be contradictory in providing an indication of the clinical course and prognosis of a particular case of DAI.

CONCLUSION

Because axonal damage is a known risk factor for neurodegeneration and dementia in the long term, early diagnosis of DAI is critical to ensure appropriate early treatment and prevention or reversal of brain damage when possible. This study showed a dichotomy of NRGN serum levels after DAI at different time intervals. These should be considered in the early diagnosis of DAI. Future clinical studies are needed to verify our results and clarify the role of changes in NRGN serum and CSF levels after DAI.

ACKNOWLEDGEMENTS

Preparation for publication of this article is partly supported by Turkish Neurosurgical Society and Prof. Dr. Cemil Tascioglu State Hospital Medical Expertise Education Board.

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