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Original Research Article

EVALUATION OF TRAUMA SCORING AND ENDOTHELIAL GLYCOCALYX INJURY IN CATS WITH HEAD TRAUMA

Kurtulus Parlak[1],*, Amir Naseri[2], Mustafa Yalcin[1], Eyup Tolga Akyol[3], Mahmut Ok[2], Mustafa Arican[1]

[1]Department of Surgery, [2]Department of Internal Medicine, Faculty of Veterinary Medicine, University of Selcuk, 42130, Konya, [3]Department of Surgery, Faculty of Veterinary Medicine, Balikesir University, 10145, Balikesir, Turkey

*Corresponding author, E-mail: kparlak@selcuk.edu.tr

Abstract: This study aim to evaluate the modified Glasgow Coma Scale (mGCS) and Animal Trauma Triage (ATT) scores, laboratory variables, and prognostic features of trauma-induced endothelial glycocalyx injury in cats with head trauma. Twenty-five cats with head trauma and 10 healthy cats were evaluated in this study. The enzyme-linked immunosorbent assay (ELISA) method was used to measure the levels of syndecan-1 and thrombomodulin in the serum of the 25 cats with head trauma (within 48 hours) and the 10 healthy cats. In addition, mGCS scores, ATT scores, laboratory values, syndecan-1 and thrombomodulin levels were compared between the cats that survived following treatment and the cats that did not survive despite treatment. Syndecan-1 and thrombomodulin levels were not statistically different between healthy cats and cats with head trauma. In the cats with head trauma, the mGCS scoring system was found to be more sensitive than the ATT scoring system. In conclusion, syndecan-1 and thrombomodulin levels did not yield statistically significant results in the cats with head trauma.

Key words: animal trauma triage; cat; head trauma; modified Glasgow coma scale; endothelial glycocalyx

Introduction

Head trauma is the most common injury in cats after extremity trauma (1). This type of trauma is commonly associated with motor vehicle accidents and high-rise syndrome (2, 3). Modified Glasgow Coma Scale (mGCS) and Animal Trauma Triage (ATT) are trauma-specific scoring systems used to classify trauma patients for prognostic purposes with quantification of injury severity (4–6). Trauma scores can facilitate the objective assessment of traumatized animals, and improve the outcome of treatments by predicting prognosis. The ATT score assesses six categories of body systems (perfusion, cardiac, respiratory, eye/muscle/integument, skeletal, and neurologic) and a scale of 0 to 3 for each category (0-slight or no injury, 3-severe injury), which contribute equally to the total score of 0 to 18 for prediction (5, 7). The mGCS was modified for veterinary use from the Glasgow Coma Scale, which has been described for humans with traumatic brain injury. This scale is based on the assessment of three categories, motor activity, brainstem reflexes, and level of consciousness. For each category, there is a scale from 1 to 6 (1-severely abnormal, 6-normal), with a lower total score indicating the more severe neurological deficits (4).

Recently, resuscitation efforts in severe trauma patients (humans, cats, dogs, etc.) have focused not only on restoring lost blood volume, but also on improving the recovery of inflammatory and coagulation responses, vascular permeability,

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and endothelial dysfunction (8, 9). In humans, head trauma and hemorrhagic shock are the most common causes of death in patients with trauma (10). In particular, hemorrhagic shock leads to systemic degradation of the endothelial glycocalyx layer, and these changes are thought to lead to traumatic endotheliopathy (EoT), a syndrome associated with high mortality (11–13). Therefore, biomarkers such as syndecan-1 and thrombomodulin have emerged for the assessment of coagulation and endothelial integrity (endothelial glycocalyx). In particular, hemorrhagic shock, sepsis, multiorgan failure, endothelial dysfunction, and damaged vascular permeability have been associated with increased morbidity and mortality (14, 15).

Overall, the purpose of this study is to investigate the mGCS and ATT scores as well as laboratory variables and markers of endothelial glycocalyx dysfunction for prognosis in cats with head trauma.

Materials and methods

Criteria for the selection of cases

Cats with a history of head injury (within 48 hours) that met clinical and neurologic examination criteria (mGCS and ATT scores) were included in the study. Delayed (over 48 hours), treated cases, and cats with polytrauma were excluded from the study.

Scoring methods

Clinical examinations of the cats with head injuries were performed by the same observer (KP) using the mGCS and the ATT scoring systems before analgesic medication was administered. On the mGCS, consciousness, brainstem reflexes, and motor activities were rated in three scoring categories: 3 to 8, “grave”; 9 to 14, “guarded”; 15 to 18, “good.” As with the ATT assessment, body systems were rated in six categories (perfusion, cardiac, respiratory, eye/muscle/integument, skeletal, and neurologic) and scored on a scale of 0 to 3 in each category (4, 5).

CT imaging

A CT scan of the head was performed within 72 hours after trauma when the patient’s condition had stabilized. The patient was premedicated with medetomidine hydrochloride (Domitor®, Zoetis) (0.08 ml/kg b.w., intramuscularly), and anesthesia was induced with propofol (Propofol %1, Fresenius Kabi) (4 - 6 mg/kg b.w., intravenously) and maintained with isoflurane (Forane®, Abbott) in oxygen via intubation tube. For the CT 120 kV, 100 mA, and 2 mm cross-sectional thickness were selected and performed in helical cranial scanning mode. CT was used for head bone assessment only.

Blood sample collection

On admission, 2 ml of blood was collected by jugular venipuncture. Depending on the clinical condition of the cases, blood analyzes were repeated during the monitoring process. One part (0.5 ml) of the collected sample was immediately used for venous blood gas analysis, and the rest for complete blood count (CBC) and biochemistry (serum) analysis. Serums were stored at – 80 °C and thawed immediately before ELISA (enzyme-linked immunosorbent assay) analysis.

Blood gases and complete blood count

Venous blood gas analysis, which includes pH, partial pressure of carbon dioxide (pCO_2), partial pressure of oxygen (pO_2), venous oxygen saturation (sO_2), lactate, sodium (Na), calcium (Ca), chloride (Cl), potassium (K), glucose, base excess (BE), and bicarbonate (HCO_3) was performed using an automated blood gas analyzer (ABL 90 Flex, Radiometer, USA). Blood counts including total leukocytes, lymphocytes, monocytes, granulocytes, erythrocytes, hematocrit (HCT), hemoglobin (Hgb), and platelets were obtained using an automated cell counter (MS4e, Melet Schlosing Laboratories, France).

Measuring syndecan-1 and thrombomodulin by the ELISA method

Serum concentrations of syndecan-1 and thrombomodulin were measured according to the manufacturer’s protocol using the feline syndecan-1 commercial sandwich ELISA kit (catalog number: MBS1603385, USA) and the feline thrombomodulin commercial ELISA kit (catalog number: MBS1603383, USA) with an ELISA reader (Biotek 800TS, BioSPX, The Netherlands). Intraassay coefficients, inter-assay coefficients, and minimum detectable concentrations were < 8%, < 10%, and 0.025 ng/ml for syndecan-1 cat, < 8%, < 10%, and 0.017 ng/ml for thrombomodulin.

Treatment protocol

The treatment protocol was applied to the cats with head trauma immediately after scoring and blood collection, and this protocol was repeated depending on the condition of the patients in the intensive care unit. The goals of the treatment protocol were based on the stimulation of circulation and oxygen delivery to vital organs. Fluid therapy with crystalloid solution 0.9% NaCl (60 ml/kg/hour, b.w., intravenously), osmotic diuretic mannitol (20% Mannitol, Polifarma) (2 g/kg b.w., intravenously, in 15 minutes), oxygen therapy (flow rate 100 ml/kg/min, intranasal or flow-by, oxygen cage), butorphanol (Butomidor®, Richter Pharma) (0.4 mg/kg b.w., subcutaneously) for analgesia, levetiracetam (Keppra®, UCB Pharma) (40 mg/kg b.w, intramuscularly) for anticonvulsant therapy, and nutrition (oral feeding or nasogastric tube feeding and esophageal feeding) were performed in cats with head trauma.

Statistical methods

To determine if the variables had a normal distribution, the Shapiro-Wilk test was used. In addition, parametric data were analyzed with the Student t-test (as mean ± standard deviation (SD)), nonparametric data were analyzed with the Mann-Whitney U test (as median (min/max)), and linear regression analysis was performed to determine the independent predictors of mortality. The prognostic value of serum endothelial biomarkers, mGCS, and hematologic variables was also evaluated using a receiver operating characteristic curve (ROC) to determine the prognostic cut-off values for best discrimination between survivors and nonsurvivors. Finally, the Kaplan-Meier survival curve from GCS was constructed. Overall, the statistical significance was $P < 0.05$, and data analysis was performed using SPSS statistical software.

Results

The animals in this study consisted of 25 cats with acute head trauma (24 mixed-breed and one British Shorthair; 14 males and 11 females; mean age, 13.4 (1-48) months) and 10 healthy cats (control group) (10 mixed-breed; 6 females and 4 males; mean age, 15.2 (8-28) months).

Of the 25 cats with head trauma, 5 cats (20%) had trauma due to high-rise syndrome and 20 cats (80%) had trauma due to motor vehicle accidents. In addition, 8 cats (32%) had severe head trauma (mGCS score 3 - 8, "grave"), 10 cats (40%) had moderate head trauma (mGCS score 9 - 14, "guarded"), and 7 cats (28%) had mild head trauma (mGCS score 15 - 18, "good"). Compared with the mGCS scores, the ATT scores ranged from 5 - 14 (mean 9) in the cats with severe trauma, from 4 - 12 (mean 7.5) in the cats with moderate trauma, and 2 - 7 (mean 4.7) in the cats with mild trauma (Table 1).

CT scan was performed in 15 / 25 cats with head trauma. The CT could not be performed in 8 cats because of their poor clinical condition and in 2 cats because their owners did not give permission. There were no abnormal findings in 2 / 15 cats. In addition, in 1 of the cats in which a CT scan was not performed, separation of the mandibular symphysis was clinically detected and treated. Regarding the CT findings of the other cats, separation of the symphysis mandible (n = 8), temporomandibular joint (TMJ) fracture (n = 5), os temporale fracture (n = 1), os zygomaticus fracture (n = 2), and separation of the palatal bone (n = 8) were observed (Table 1). There were no complications related to anesthesia during the CT scans.

Finally, 12 of the 25 cats with head trauma were discharged after treatment (the average treatment time for discharged cats is 96 hours), whereas the remaining 13 cats died (no cat was euthanized). In this context, hematologic values, trauma scores (mGCS, ATT), and endothelial glycocalyx layer data (syndecan-1, thrombomodulin) were statistically compared between non-surviving and surviving cats (Figure 1) (Table 2).

Linear regression analysis showed that mGCS, K^+, HCO_3, WBC, and Hb were independent risk factors for mortality in the head trauma group, whereas ROC analysis for the benefit of mGCS in discriminating between surviving and non-surviving cats yielded an area under the curve of 0.76 (p = 0.028, 95% CI = 0.569-0.950) (Figure 2). Furthermore, the optimal cut-off point of 14.50 for the mGCS corresponded to a sensitivity of 76% and a specificity of 70% for predicting

Table 1: Trauma scores and findings on cases

Case	Trauma Type	CT Results	mGCS	ATT	Survivors/ Non-survivors
Cat 1	HRS	TMJ Fracture (Left) Os zygomaticus fracture	14	8	N
Cat 2	MVA	CT not applied	17	2	N
Cat 3	MVA	TMJ Fracture (Right)	16	7	N
Cat 4	MVA	CT not applied	5	9	Y
Cat 5	MVA	CT not applied	6	9	N
Cat 6	MVA	CT not applied	3	5	N
Cat 7	MVA	CT not applied	4	6	N
Cat 8	MVA	Separation of the symphysis mandible	18	7	Y
Cat 9	MVA	TMJ fracture (Left) Separation of the symphysis mandible	16	5	Y
Cat 10	MVA	Separation of the palatal bone	16	3	Y
Cat 11	MVA	TMJ Fracture (Right) Separation of the symphysis mandible Separation of the palatal bone	3	11	N
Cat 12	MVA	Os temporale fracture Separation of the palatal bone	12	5	Y
Cat 13	MVA	Separation of the symphysis mandible Separation of the palatal bone	15	7	Y
Cat 14	HRS	Different structure not seen	14	9	N
Cat 15	MVA	Separation of the symphysis mandible	14	9	N
Cat 16	MVA	Separation of the symphysis mandible Separation of the palatal bone	10	6	Y
Cat 17	MVA	TMJ fracture (Right) Separation of the palatal bone	11	7	N
Cat 18	MVA	Os zygomaticus fracture Separation of the symphysis mandible Separation of the palatal bone	5	10	N
Cat 19	HRS	Different structure not seen	13	8	N
Cat 20	MVA	Separation of the palatal bone	9	4	Y
Cat 21	MVA	CT not applied	6	7	Y
Cat 22	HRS	CT not applied	18	2	Y
Cat 23	MVA	CT not applied	13	7	Y
Cat 24	MVA	CT not applied	13	7	Y
Cat 25	HRS	CT not applied	4	14	N

HRS: High-Rise Syndrome, TMJ: Temporomandibular Joint, MVA: Motor vehicle accidents, mGCS: Modified Glasgow Coma Scale, ATT: Animal Trauma Triage, Y: Survivors, N: Non-survivors

mortality. Finally, a probability curve for the mGCS score showed a 72% probability of nonsurvival at a score of 6 and a 56% probability of non-survival at a score of 13, whereas a mentation score of 3 showed a 92% probability of nonsurvival (Figure 3).

Discussion

The primary causes of head injuries are traffic accidents, followed by high-rise syndrome (3). Of the 25 cats with head trauma in the present study, 80% were due to motor vehicle accidents, whereas 20% were due to high-rise syndrome, which is consistent with the results of previous studies.

Some researchers have evaluated different scoring systems, such as the mGCS and ATT scoring systems, as prognostic indicators of trauma in cats and dogs (4, 7, 16). For instance, Lapsley et al (7) studied the mGCS and ATT scoring systems in 711 cats with trauma.

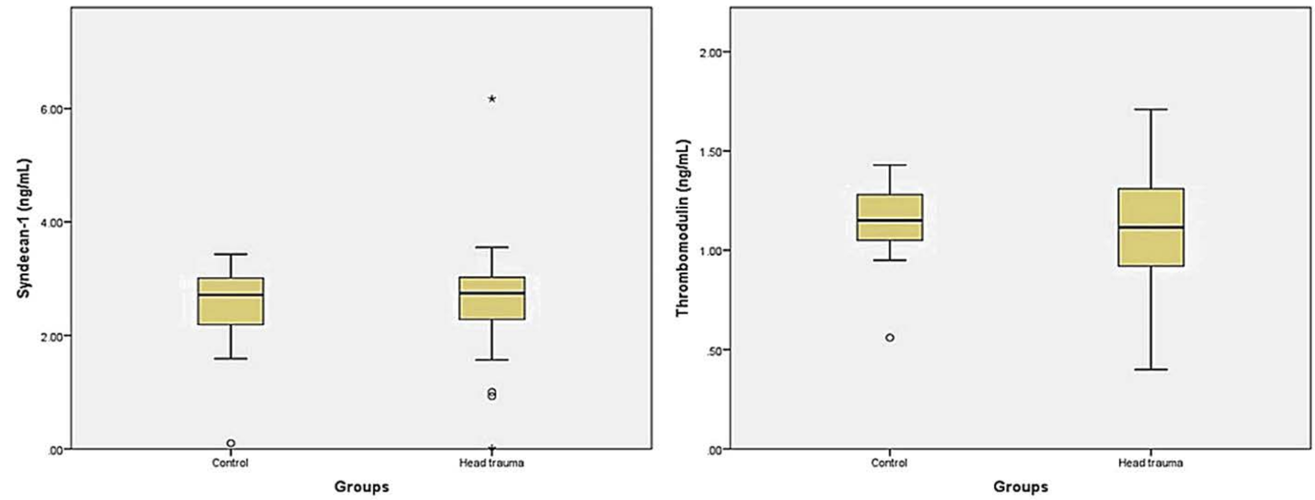

Figure 1: Comparison of the syndecan-1 (left) and thrombomodulin (right) levels of healthy cats and cats with head trauma

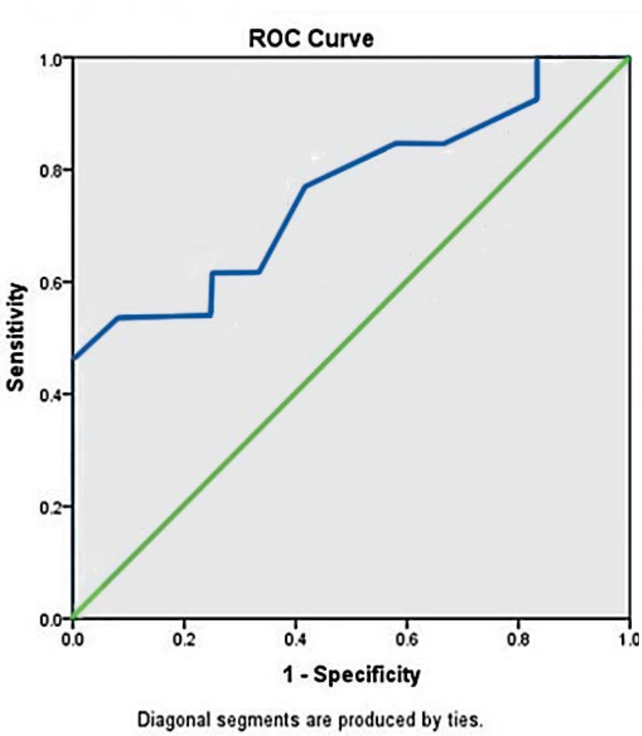

Figure 2: Receiver operating characteristic curve (ROC) analysis for the utility of the modified Glasgow Coma Scale to discriminate between surviving and nonsurviving cats yielded an area under the curve of 0.76 (p = 0.028, 95% CI = 0.569 - 0.950)

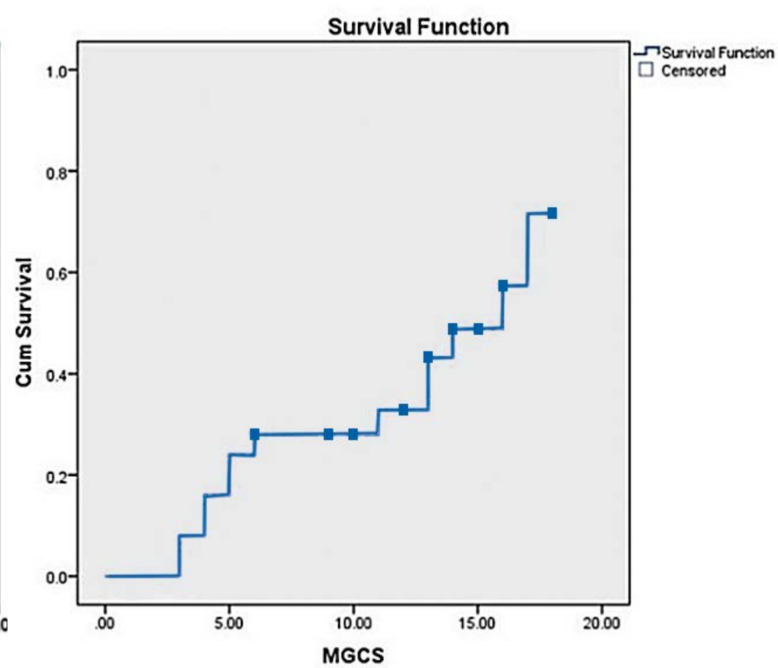

Figure 3: A probability curve for the modified Glasgow Coma Scale score showed a 72% probability of nonsurvival at a score of 6 and a 56% probability of nonsurvival at a score of 13, whereas a mentation score of 3 showed a 92% probability of nonsurvival

However, when they limited the study to patients with head trauma, they found that there was no significant difference in differential capacity between the two scoring systems.

In a related study, Sharma and Holowaychuk (16) performed prognostic assessments of 72 dogs with head trauma and found that both scoring systems, particularly the mGCS, had prognostic value. In addition, Platt et al (4) reported that the mGCS can be evaluated as prognostic data in dogs with head trauma. In the present study, when the mGCS and ATT scores of the non-surviving and

Table 2: Mean trauma scores (mGCS, ATT), endothelial glycocalyx layer data (syndecan-1, thrombomodulin), and hematologic values were statistically compared between nonsurviving and surviving cats

Parameter	Survivors (n: 12)	Non-survivors (n: 13)	P-value
mGCS	14 (6 - 18)	6 (3 - 17)	0.026
ATT	6 (2 - 12)	8 (2 - 14)	0.110
Syndecan-1 (ng/ml)	2.49 ± 0.94	2.59 ± 1.34	0.827
Thrombomodulin (ng/ml)	1.14 ± 0.30	1.02 ± 0.29	0.319
pH	7.32 ± 0.49	7.26 ± 1.12	0.076
pCO_2 (mmHg)	35.02 ± 5.90	35.56 ± 5.73	0.818
pO_2 (mmHg)	36.54 ± 6.18	34.06 ± 4.01	0.255
sO_2 (%)	47.62 ± 14.17	44.38 ± 8.64	0.503
cK^+ (mmol/L)	3.72 ± 0.58	3.19 ± 0.68	0.047
cNa^+ (mmol/L)	159.58 ± 3.08	159.53 ± 9.68	0.988
cCa^+ (mmol/L)	0.87 ± 0.19	0.81 ± 0.22	0.452
cCl^- (mmol/L)	120 (116 - 125)	121 (92 - 181)	0.205
cGlu (mg/dL)	209.58 ± 52.64	212.76 ± 66.50	0.895
cLac (mmol/L)	3.00 ± 1.75	2.13 ± 1.34	0.182
cBase (Ecf) (mmol/L)	-7.70 ± 1.55	- 10.21 ± 4.05	0.055
$cHCO_3^-$ (mmol/L)	17.90 ± 1.13	16.05 ± 2.63	0.034
WBC (cells/ml)	26.36 ± 11.65	15.79 ± 9.22	0.021
LYM (cells/ml)	7 (1 - 18)	5 (2 - 15)	0.247
MON (cells/ml)	1 (0.14 - 12)	0.99 (0.51 - 4)	0.437
GRA (cells/ml)	14.12 ± 8.28	8.67 ± 7.80	0.105
RBC ($x10^3$ cells/ml)	10.66 ± 1.93	8.72 ± 2.72	0.051
Hct (vol%)	44.07 ± 9.65	36.12 ± 11.26	0.070
Hgb (g/dl)	12 (8 - 45)	10 (7 - 16)	0.022
Platelets (cells/ml)	199.58 ± 101.84	214.76 ± 76.02	0.679

surviving cats were compared, it was found that the mGCS was statistically more prominent in head trauma in terms of prognosis. It was also suggested that the lack of statistical prognostic value of the ATT system was since this study focused only on cats with isolated head trauma and not on cats with polytrauma. It is important to note that the ATT scoring system generally assesses all body systems, including the musculoskeletal system, whereas the mGCS provides a more specific assessment of the central nervous system.

In general, CT scans are used to diagnose hematomas, contusions, hernias, and cerebral ischemia, especially fractures in patients with head trauma (17, 18). It has also been used in the determination of craniomaxillofacial fractures in cats with head trauma. Recent studies report that mandibular fractures, particularly the separation of the mandibular symphysis and TMJ fractures, are the most common injuries diagnosed with CT in cats after head trauma (19, 20). In addition, Tundo et al (20) stated that fractures involving the skull in cats after head trauma had a common and predictable distribution pattern in the midface (nose, maxilla, intermaxillary suture, orbit, nasopharynx, and zygomatic arch), with a high incidence of TMJ fractures. According to the CT scans in this study, the most common injuries were the separation of the mandibular symphysis, separation of the palatal bone, and TMJ fractures. The results are consistent with those of recent studies (19, 20). It is important to point out that one of the main problems that limited our CT imaging method was the inadequacy of the older generation of equipment, which prevented us from scanning thinner sections and obtaining detailed views of small structures.

Some retrospective studies have been performed to determine the association between the severity of injury and hyperglycemia in cats and dogs with head trauma. Thus, some studies have indicated that cats and dogs with head trauma may have hyperglycemia, the extent of which is related to the severity of head trauma (16, 21). However, in the present study, no statistically significant difference was found between hyperglycemia and mortality, based on the statistical analyses between non-surviving and surviving cats with head trauma. However, blood glucose levels were found to be higher than normal in the cats with head trauma.

In this study, Hgb, HCO_3, and K^+ levels decreased below normal values on laboratory examination of non-surviving cats at the time of admission. Their white blood cell counts, although at normal levels, were lower than those of the surviving cats. In addition, a decrease in venous pO_2 was observed in all cats with head trauma, including both non-surviving and surviving cats. Previous studies have shown that such a decrease, especially in dogs with head trauma, is due to impaired hemodynamic stability, which may lead to secondary brain damage (16). According to the data of the present study, this situation may also occur in cats with head trauma.

Previous studies have shown an association between the detachment of glycocalyx components (syndecan-1 and thrombomodulin) and increased morbidity and mortality (22, 23). A previous study by Albert et al (24) examined the effects of endothelial damage on mortality by focusing on syndecan-1 and thrombomodulin levels in human patients with a head injury. They found that there was no significant increase in syndecan-1 levels associated with traumatic brain injury (TBI), which is a determinant of endothelial glycocalyx damage, and no significant change in thrombomodulin levels. However, they found a significant association between increased syndecan-1 levels and mortality (24). In a related study, Rodriguez et al (25) examined endothelial glycocalyx dysfunction using syndecan-1 and thrombomodulin levels in isolated TBI, polytraumatic TBI, and TBI-free trauma patients. They determined that syndecan-1 levels were highest in the polytraumatic TBI group, whereas they were lowest in the isolated TBI group. They also pointed out that thrombomodulin levels, although higher than normal, were similar in the three groups. In addition, the isolated TBI patients reported experiencing less glycocalyx destruction, as measured by their circulating syndecan-1 levels. Again, increased syndecan-1 concentrations were associated with increased 72 hours mortality in the isolated TBI patients (25).

Because there have been no previous studies of syndecan-1 and thrombomodulin levels in cats with head trauma, the results of the present study were compared with those of human studies. According to studies, the syndecan-1 levels were increased in the endothelial glycocalyx damage of patients with head trauma, and this was more strongly associated with mortality than the increase in thrombomodulin levels. However, in our study, there was no statistically significant result related to syndecan-1 and thrombomodulin levels in the cats that did not survive after head trauma. Therefore, the results of this study differ from those of human studies and the use of different biomarkers related to endothelial glycocalyx injury may lead to more efficient results.

Our study had several limitations: 1) the study population was relatively small, and reevaluation of the hypothesis of endothelial glycocalyx damage in larger sample populations is warranted. 2) Histopathological examinations were not performed on cats that did not survive after head trauma.

Conclusion

This was the first study to perform prognostic assessments based on the mGCS and ATT scoring systems and endothelial glycocalyx layers in cats with head trauma. Overall, no statistically significant difference was found between the syndecan-1 and thrombomodulin levels of the healthy cats and those of the cats with head trauma. In addition, linear regression analysis showed that the mGCS, potassium, bicarbonate, white blood cell, and hemoglobin levels were independent mortality factors in the head trauma group. Although the mGCS and ATT scoring systems are the most common scoring systems used to assess cats with trauma, the former is believed to be one step ahead of the latter. However, the markers syndecan-1 and thrombomodulin were not found to be useful for prognosis or choice of treatment options in cats with head trauma.

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VREDNOTENJE SISTEMA OCENJEVANJA TRAVME IN POŠKODB ENDOTELIJSKEGA GLIKOKALIKSA PRI MAČKAH S POŠKODBO GLAVE

K. Parlak, A. Naseri, M. Yalcin, E. T. Akyol, M. Ok, M. Arican

Izvleček: Namen te študije je bil ovrednotiti spremenjeno glasgowsko lestvico kome (angl. Glasgow Coma Scale, GCS) in ocene ATT (Animal Trauma Triage), laboratorijske spremenljivke in prognostične značilnosti s travmo povzročene poškodbe endotelijskega glikokaliksa pri mačkah s poškodbo glave. V študijo je bilo vključenih 25 mačk s poškodbo glave in 10 zdravih mačk. Ravni sindekana-1 in trombomodulina v serumu 25 mačk s poškodbo glave (v 48 urah) in 10 zdravih mačk smo merili z encimsko imunoadsorpcijsko preiskavo (ELISA). Ocene mGCS, ocene ATT, laboratorijske vrednosti ter ravni sindekana-1 in trombomodulina smo primerjali med mačkami, ki so po zdravljenju preživele, in mačkami, ki kljub zdravljenju niso preživele. Vrednosti sindekana-1 in trombomodulina se med zdravimi mačkami in mačkami s poškodbo glave niso statistično razlikovale. Pri mačkah s poškodbo glave se je sistem točkovanja mCGS izkazal za občutljivejšega od sistema točkovanja ATT. Zaključili smo, da vrednosti sindekana-1 in trombomodulina pri mačkah s poškodbo glave niso statistično pomembne.

Ključne besede: triaža poškodb živali; mačka; poškodba glave; spremenjena glasgowska lestvica kome; endotelijski glikokaliks