Ischemia Modified Albumin Levels Following Mild Closed Head Trauma in the Rat

Hafif Kapalı Kafa Travmasına Maruz Kalmış Ratlarda İskemi Modifiye Albumin Düzeyleri

Tuncay ÇOLAK, MD,[a]
Can DUMAN, MD,[b]
Belgin BAMAÇ, MD,[a]
Adem AYDIN, MD,[c]
Aydın ÖZBEK, MD,[a]
Kürşat YILDIZ, MD,[d]
Ümit BİÇER, MD[e]

Departments of
[a]Anatomy,
[b]Biochemistry,
[c]Orthopedics and Traumatology,
[d]Pathology,
[e]Forensic Medicine,
Kocaeli University Faculty of Medicine, Kocaeli

Geliş Tarihi/*Received:* 25.02.2009
Kabul Tarihi/*Accepted:* 31.08.2009

This study was presented as a poster at XXI[th] Congress of the International Academy of Legal Medicine, in 28-30 May 2009.

Yazışma Adresi/*Correspondence:*
Tuncay ÇOLAK, MD
Kocaeli University Faculty of Medicine, Departments of Anatomy, Kocaeli, TÜRKİYE/TURKEY
tuncayc@kocaeli.edu.tr

ABSTRACT Objective: Mild traumatic brain injury (MTBI) is one of the most frequent neurological damage types in which complex physiological and biochemical events occur in traumatized area. Regional ischemia can occur in the acute phase after trauma. Recently, both clinical and experimental studies have shown that ischemia modified albumin (IMA) is one of the most promising blood markers of ischemia. In this study, whether serum IMA values can be used in rats as a marker for intracranial ischemia caused by trauma is investigated. **Material and Methods:** Wistar-albino adult rats were utilized in this study. Rats were exposed to MTBI by using a trauma device. Frontal and parietal brain tissues were taken for histopathological investigation. Serum IMA values of traumatised and control rats were measured with the ACB (albumin cobalt binding) test. **Results:** While the absolute IMA values were 0.576 ± 0.048 for trauma group, it was identified as 0.568 ± 0.055 for control group. There was no statistically significant difference between trauma and control groups in the mean value of the IMA. In our histopathological analysis, necrosis associated with hypoxia in the outer layers of brain cortex as a result of the trauma applied to rats, and neuron damage in the inner layers of the cortex were observed. In addition, red neurons were detected in the brain cortex as a result of early stage neuron damage associated with ischemia. **Conclusion:** We found that there is no significant increase in IMA levels in rats with trauma suggesting that this biomarker would not be useful in detecting early ischemic changes in MTBI.

Key Words: Rats; craniocerebral trauma

ÖZET Amaç: Hafif travmatik beyin hasarı (HTBH) en sık görülen nörolojik hasarlardan biridir ve travmatize alanda kompleks fizyolojik ve biyokimyasal olaylar oluşturur. Bölgesel iskemi travmadan sonraki akut fazda görülmektedir. Son zamanlarda klinik ve deneysel çalışmalar iskemi modifiye albumin (IMA)'in, iskemide en umut verici kan belirteçlerinden biri olduğunu göstermiştir. Çalışmada ratlarda serum IMA değerlerinin travma sonucu oluşan kafaiçi iskemide belirteç olarak kullanılıp kullanılamayacağı araştırıldı. **Gereç ve Yöntemler:** Araştırmada Wistar-albino türü erişkin ratlar kullanıldı. Ratlar travma düzeneği kullanılarak HTBH'a maruz bırakıldı. Histopatalojik araştırma için frontal ve parietal beyin dokuları alındı. Travmatize ve kontrol ratların serum IMA değerleri albumin kobalt bağlama (ACB) testi ile ölçüldü. **Bulgular:** Travma grubuna ait IMA değeri 0.576 ± 0.048 iken kontrol grubunda bu değer 0.568 ± 0.055 olarak saptanmıştır. Travma ve kontrol gruplarının IMA değerleri arasında istatistiksel olarak anlamlı bir farklılık bulunmadı. Yaptığımız histopatolojik incelemelerde, ratlara uyguladığımız travma sonucu beyin korteksine ait kesitlerde en dışta hipoksiye bağlı nekroz, iç tabakada ise nöronlarda zedelenme gözlemlendi. Ayrıca iskemiye bağlı erken dönem nöron zedelenmesi sonucu beyin korteksinde kırmızı nöronlar saptandı. **Sonuç:** HTBH sonrası ratlar ile kontrol grubu arasında IMA değerlerinde anlamlı bir farklılık olmadığı için, IMA'nın, kafa içi iskemide bir belirteç olarak kullanılamayacağı sonucuna varıldı.

Anahtar Kelimeler: Rat; kafa travması

Copyright © 2010 by Türkiye Klinikleri

Turkiye Klinikleri J Med Sci 2010;30(4):1171-6

Traumatic brain injury (TBI) is a major cause of death and disability in both children and elderly people. In the literature, TBI is commonly classified as mild, moderate or severe based on the Glasgow Coma Score (GCS) at first examination.[1-4] Mild and severe TBI is considered to have the same underlying neuro-pathology, namely diffuse axonal and vascular injury occurring to a various extent in the parasagittal deep white matter, spreading from cortex to brainstem.[2] Mild traumatic brain injury (MTBI) can be accompanied by facial and skull fractures. The presence of a skull fracture in MTBI implies an increased risk of intracranial injuries.[1,5] Small vessel injury can lead to haemorrhagic diffuse axonal injury lesions predominantly in the gray matter, owing to better vascularization. Injury to larger blood vessels result in intracerebral haemorrhages.[6] Following a contusion or haemorrhage, blood extends into adjacent cortex where neurons undergo secondary necrosis due to ischemia.[7]

Cerebral ischemia can occur following MTBI and early ischemia after MTBI may be a great influential factor determining neurological outcome. Nabika et al reported ischemia of the internal capsule after mild head injury in a 1-year-old boy.[8] In an experimental study of Jenkins et al., it is proposed that mild mechanical injury can potantiate selective ischemic hippocampal neuronal necrosis in the absence of overt axonal injury.[9] There appears to be an interaction between the ischemia and neuronal injury following MTBI. Son et al measured N-acetylaspartate/Creatine ratio and lactate signal on in vivo proton magnetic resonance spectroscopy in order to define metabolic brain changes associated with MTBI in patients with regional brain contusion and 13-15 of initial GCS. Cell loss and ischemic damage have been indicated in region of interest at early stage.[10]

Assessment of MTBI is problematic for a number of reasons. First, the standard protocol used to assess TBI severity and plan rehabilitation is dominated by CT, MRI and neurobehavioral procedures. While these procedures may be effective for moderate to severe injury, they may be less useful for the assessment of MTBI.[11] The authors comment that examinations as CT, MRI, EEG and routine neurological evaluations may be normal.[12]

There appears to be an interaction between the posttraumatic cerebral ischemia and secondary neuronal injury. The early identification of ischemia is an important predictor of long-term outcome. A rapid blood test to diagnose ischemia would be of greatest utility after MTBI. Ischemia modified albumin (IMA) measurement referred as albumin cobalt binding test (ACB) is a new biomarker of cardiac ischemia, skeletal muscle ischemia and cerebral ischemia.[13-15] To investigate the clinical utility of IMA as a serum marker for MTBI, we attempted to evaluate the role of IMA in rats with closed head MTBI.

MATERIAL AND METHODS

To determine ischemic events following MTBI we used the animal model of closed head injury. The severity of brain injury can easily be controlled in this model through the adjustment of the height and mass of the weight used for injury.[16] Rats were provided by the Experimental Medical Research Center of the Medical Faculty of Kocaeli University and the Animals Ethics Commitee approved the experimental protocol. Closed traumatic brain injury was made by weight drop according to an experimental model described by Marmarou et al.[17] On the other hand Ucar et al[18] have made some modifications on the technique of this model for an ideal MTBI model formation. We preferred their modified system to make MTBI in this study.

ANIMALS

In the present study, 30 male adult Wistar-albino rats (200 to 250 g) were housed individually under controlled environmental conditions (12 hours light/12 hours dark cycle). Animals were divided in two groups. Fifteen rats underwent MTBI and, 15 rats served as control animals. Initially rats were anesthetized with ether. Animals were able to breathe spontaneously throughout the procedures. In control animals, all experimental procedures were conducted but the rats were not traumatized.

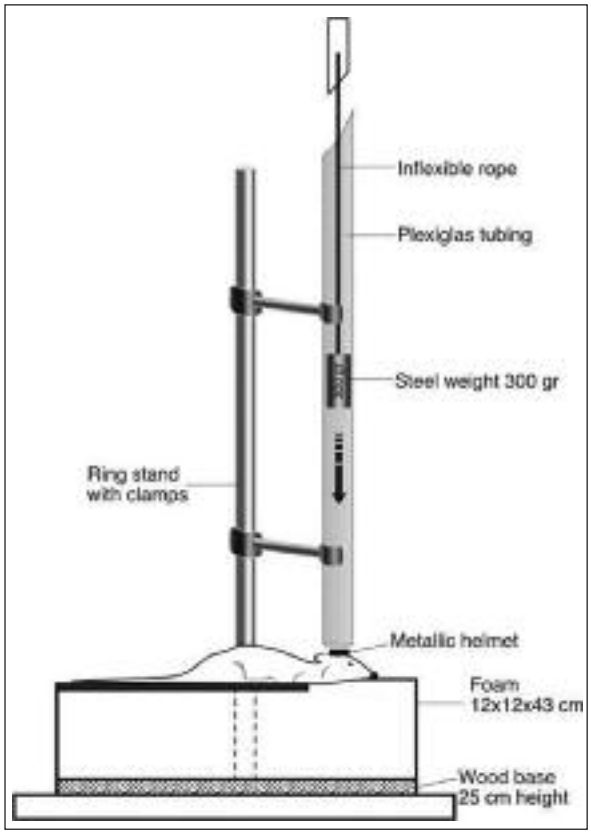

FIGURE 1: Diagram of trauma.

TABLE 1: Mean IMA levels of trauma and control groups (mean ± SD).

	Trauma group (n:15)	Control group (n:15)	p
IMA (ABSU)	0.576 ± 0.048	0.568 ± 0.055	0.443

TRAUMA DEVICE AND INDUCTION OF MILD TRAUMATIC BRAIN INJURY

The trauma device consisted of a unique column of a plexiglass tube with a freely falling steel weight by gravity onto a metallic helmet fixed on the skull vertex of the rat by bonewax. The steel weight falls through a 1m vertical section of the plexiglass tube held in place with a ring stand (Figure 1).[18] We have employed an inflexible rope for tying the weight to prevent undesirable movements after the first impact as recommended by Ucar et al.[18] In addition, the tube was perforated at 3 cm intervals for minimizing the effects of frictional forces. The animal was placed in prone position on a foam bed. A 3mm thick stainless-steel disc was served as the helmet. Before the force was applied a midline skin incision was performed and skin was reflected to expose the skull. The metallic disc was fixed to the central portion of the skul. A cylindrical weight was placed in the tip of the tube and the opening of the tube was centered over the helmet directly on rat's skull. A 300 g weight was then dropped from the top of the tube from a height of exactly 1 m. The animals were removed after the impact and they were moved from the device to their cage. Rats were kept under observation for 1 hour. Then animals were anesthetized again and the blood was taken from right atrium of heart. Blood samples were drawn from all animals.

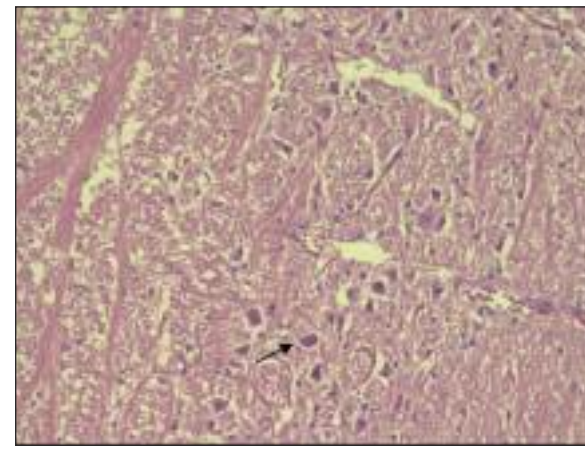

FIGURE 3: Red neurons were seen in early stage of ischemia by haematoxylin and eosin staining (HE, x400).

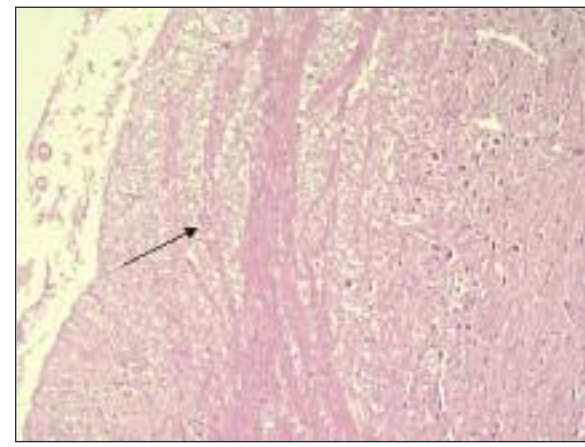

FIGURE 2: Neuron damage in inner layer of cortex and neuronal necrosis were observed in outer layers of cortex of the rat brains following cerebral hypoxia (HE, x100).

LABORATORY ANALYSIS

The IMA was studied in blood from right atrium of the heart. The blood samples were taken into plain tubes containing no preservatives, the clot formation were allowed for 30 minutes and the blood samples were centrifuged at 3000 g for 10 minutes to separate the sera. Then, the obtained serum samples were subjected to measurement immediately. ACB test was analyzed according to the method defined by Bar-Or et al.[13] In this method, 200 mL serum was added to the water solution of 50 mL %0.1 (w/v) cobalt chloridine (Sigma; $CoCl_2.6H_2O$). It was mixed gently and waited for 10 minutes for sufficient cobalt-albumin binding. Then, 50 mL dithiothreitol (DTT) (Sigma; 1.5 mg/ml H_2O) was added as a colorizing agent. After waiting for two minutes 1.0 ml 0.9% NaCl was added to stop the cobalt binding process of albumin. Afterwards, the absorbance was measured through spectrophotometer at 470 nm (Shimadzu, model UV160U). Sample blank without DTT were used as blind. The results were reported as absorbance units (Absorbance Units, ABSU).[13]

LIGHT MICROSCOPIC EXAMINATIONS

Frontal and parietal brain tissues were taken for histopathological investigation. The brains were removed, postfixed in 4% paraformaldehyde overnight at 4 °C, embedded in paraffin wax, and then serial coronal 5- mm sections were cut and subjected to standard hematoxylin and eosin (HE) staining. Briefly, the sections were dewaxed with xylene and rehydrated through a series of graded alcohols. They were mounted on slides and stained with hematoxylin for 3 minutes and rinsed in tap water and stained with eosine for 2 minutes. They were dehydrated and cleared with xylene. Slides were examined through light microscopy by a pathologist.

STATISTICAL ANALYSIS

All data were presented as mean ± SD. Statistical analysis was performed using SPSS 15.0 software program. We compared numerical variables between two groups using the two-tailed Mann-Whitney U test. Statistical significance was assumed at a level of $P< 0.05$.

RESULTS

Measured mean IMA levels are presented in Table 1. The absolute IMA values were 0.576 ± 0.048 for trauma group and 0.568 ± 0.055 for control group. There was no statistically significant difference between trauma and control groups in the mean value of the IMA (p= 0.443).

In trauma group, light microscopic examinations revealed neuron damage in inner layer of cortex and neuronal necrosis were observed in outer layers of cortex of the rat brains following cerebral hypoxia (Figure 2). Red neurons were seen in early stage of ischemia by haematoxylin and eosin staining (Figure 3).

DISCUSSION

Recent studies have demonstrated that IMA levels increase in the acute ischemic stroke,[19] acute phase of cerebrovascular diseases[15] and after cardiac ischemia.[19,20] However, the usefulness of IMA measurement in clinical practice and diagnosis of certain types of cerebrovascular event is still uncertain.[15] We therefore designed a study in rats with MTBI which is IMA level predictive of an intracranial ischemia. Results of our study showed that there is no significant increase in IMA levels in rats with trauma suggesting that this biomarker may not be useful in detecting early ischemic changes in MTBI.

During acute ischemic condition, the metal binding capacity of albumin for transition metals, such as copper, nickel and cobalt is reduced, generating a metabolic variant of the protein, commonly known as IMA.[15] Although the precise mechanism for IMA production is still not identified, ischemia may lead to a site-specific modification of the N terminus of albumin. In intact human albumin, transition metals such as cobalt can bind tightly to the exposed N terminus of albumin.[19,21] During ischemia/reperfusion, modifications altering the binding capacity of albumin for cobalt may ocur as the result of acidosis, reduced oxygen tension and generation of free radicals.[13,19] Free radical production increases in stroke and play a role in the pathogenesis of the histological damage during bra-

in ischemia.[22] It has been suggested that IMA is in fact a marker of oxidative stress, while conditions associated with raised IMA can be related to other markers of oxidative stress.[23,24] It is suggested that during the oxidative stress occuring in acute stroke, the combined production of reactive oxygen species with free radicals and the passage through an impaired brain-blood barrier can explain in part the IMA formation.[19] In a recent study by Tavazzi et al the occurence of oxidative and nitrosative stresses in rats undergoing recurrent MTBI delivered with increasing time intervals was investigated.[25] They showed the remarkable negative contribution of reactive oxygen species overproduction and activation of inducible nitric oxide synthase in recurrent MTBI. These effects were maximal when MTBIs were spaced by 3 days so it can be inferred that occurence of a second MTBI induces sustained oxidative and nitrosative stresses. Although one might have expected an increase in IMA levels in rats with trauma, there was no significant differences between trauma and control group in the present study. Since we applied 300 g-1 m impact, it seems that this is not enough to produce an oxidative stress. It can be said that significant changes in IMA levels can be expected in more severe head injury models.

It has been speculated that the mild injuries typically result in axonal damage found within brain parenchyma showing no other signs of neuronal or vascular change. On the other hand moderate to severe injuries frequently result in vascular damage.[11,26] However, the pathophysiologic changes taking place immediately after the head trauma are not yet fully understood. The severity of the primary impact to the brain is an important factor in the functional and pathological consequences of TBI.[27] Some authors have observed increased IMA levels in acute stroke.[19,28] Abboud et al[17] showed that IMA is a biomarker for early identification of acute ischemic stroke. In the present study, applied impact which we used for head injury model might not be as severe as the ischemia that occurs during acute ischemic stroke. They reported increase in IMA levels in brain infarctions, whereas no change in brain hemorrhages during the first 24h. In their study, the serum samples for IMA were obtained from patients within the first 3h of neurological deficit onset. A complex chemical cascade of biochemical events occur during the first minutes after MTBI. We obtained the blood samples 1 hour later following trauma. It would be better if we take them immediately because active ischemia might have to increase IMA, but the hemoconcentration mechanism predominates and it is resulted in decreased IMA concentration

Further studies are needed to define the role of IMA in diagnosis of ischemia in various ischemic states in humans. Since susceptibility of cells to ischemia may vary from one organ to another, it would be critical to determine the optimal IMA levels for the diagnosis of ischemia in various organs, especially the heart and brain.[28] We concluded that IMA values shortly after closed MTBI cannot be used to detect intracranial ischemia, contrary to expectations. IMA may show a relationship with the pattern of traumatic brain injury and may be significantly higher in more severe forms of traumatic brain injury.

Acknowledgment

We would like to thank I.Tahir Erdal for his help in drawing figure.

REFERENCES

1. De Kruijk JR, Twijnstra A, Leffers P. Diagnostic criteria and differential diagnosis of mild traumatic brain injury Brain Inj 2001;15(2):99-106.
2. Alexander MP. Mild traumatic brain injury: pathophysiology, natural history, and clinical management. Neurology 1995;45(7):1253-60.
3. Bekman ZM. [Head trauma]. Turkiye Klinikleri J Int Med Sci 2007;3(5):35-43.
4. Akdemir G. [Trauma scoring and glasgow coma scale]. Turkiye Klinikleri J Neurosurg 2008;1(1):6-11.
5. Murshid WR. Management of minor head injuries: admission criteria, radiological evaluation and treatment of complications. Acta Neurochir (Wien) 1998;140(1):56-64.
6. Voller B, Auff E, Schnider P, Aichner F. To do or not to do? Magnetic resonance imaging in mild traumatic brain injury. Brain Inj 2001;15(2):107-15.

7. Gennarelli TA, Graham DI. Neuropathology of the Head Injuries. Semin Clin Neuropsychiatry 1998;3(3):160-75.
8. Nabika S, Kiya K, Satoh H, Mizoue T, Oshita J, Kondo H. Ischemia of the internal capsule due to mild head injury in a child. Pediatr Neurosurg 2007;43(4):312-5.
9. Jenkins LW, Moszynski K, Lyeth BG, Lewelt W, DeWitt DS, Allen A, et al. Increased vulnerability of the mildly traumatized rat brain to cerebral ischemia: the use of controlled secondary ischemia as a research tool to identify common or different mechanisms contributing to mechanical and ischemic brain injury. Brain Res 1989;477(1-2):211-24.
10. Son BC, Park CK, Choi BG, Kim EN, Choe BY, Lee KS, Kim MC, Kang JK. Metabolic changes in pericontusional oedematous areas in mild head injury evaluated by 1H MRS. Acta Neurochir 2000;76(Suppl):13-6.
11. Gaetz M. The neurophysiology of brain injury. Clin Neurophysiol 2004;115(1):4-18.
12. Voller B, Benke T, Benedetto K, Schnider P, Auff E, Aichner F. Neuropsychological, MRI and EEG findings after very mild traumatic brain injury. Brain Inj 1999;13(10):821-7.
13. Bar-Or D, Lau E, Winkler JV. A novel assay for cobalt-albumin binding and its potential as a marker for myocardial ischemia-a preliminary report. J Emerg Med 2000;19(4):311-5.
14. Roy D, Quiles J, Sharma R, Sinha M, Avanzas P, Gaze D, Kaski JC. Ischemia-modified albumin concentrations in patients with peripheral vascular disease and exercise-induced skeletal muscle ischemia. Clin Chem 2004;50(9):1656-60.
15. Gunduz A, Turedi S, Mentese A, Altunayoglu V, Turan I, Karahan SC, et al. Ischemia-modified albumin levels in cerebrovascular accidents. Am J Emerg Med 2008;26(8):874-8.
16. Morales DM, Marklund N, Lebold D, Thompson HJ, Pitkanen A, Maxwell WL, et al. Experimental models of traumatic brain injury: do we really need to build a better mousetrap? Neuroscience 2005;136(4):971-89.
17. Marmarou A, Foda MA, van den Brink W, Campbell J, Kita H, Demetriadou K. A new model of diffuse brain injury in rats. Part I: Pathophysiology and biomechanics. J Neurosurg 1994;80(2):291-300.
18. Ucar T, Tanriover G, Gurer I, Onal MZ, Kazan S. Modified experimental mild traumatic brain injury model. J Trauma 2006;60(3):558-65.
19. Talwalkar SS, Bon Homme M, Miller JJ, Elin RJ. Ischemia modified albumin, a marker of acute ischemic events: A pilot study. Inn Clin Lab Sci 2008;88(2):132-7.
20. Bar-Or D, Winkler JV, Vanbenthuysen K, Harris L, Lau E, Hetzel FW. Reduced albumin-cobalt binding with transient myocardial ischemia after elective percutaneous transluminal coronary angioplasty: a preliminary comparison to creatine kinase-MB, myoglobin, and troponin I. Am Heart J 2001;141(6):985-91.
21. Sadler PJ, Tucker A, Viles JH. Involvement of a lysine residue in the N-terminal Ni2+ and Cu2+ binding site of serum albumins. Comparison with Co2+, Cd2+ and Al3+. Eur J Biochem 1994;220(1):193-200.
22. Zini I, Tomasi A, Grimaldi R, Vannini V, Agnati LF. Detection of free radicals during brain ischemia and reperfusion by spin trapping and microdialysis. Neurosci Lett 1992;138(2):279-82.
23. Collinson PO, Gaze DC. Ischaemia-modified albumin: clinical utility and pitfalls in measurement. J Clin Pathol 2008;61(9):1025-8.
24. Seneş M, Kazan N, Coşkun O, Zengi O, Inan L, Yücel D. Oxidative and nitrosative stress in acute ischaemic stroke. Ann Clin Biochem 2007;44(Pt 1):43-7.
25. Tavazzi B, Vagnozzi R, Signoretti S, Amorini AM, Belli A, Cimatti M, et al. Temporal window of metabolic brain vulnerability to concussions: oxidative and nitrosative stresses--part II. Neurosurgery 2007;61(2):390-5.
26. Yağmur Y, Uğur M, Kara İH, Güloğlu C, Aldemir M, Kiraz M. [Evaluation of trauma scores, and AT-III, blood trombocyte count, APTT and PT in patients with multiple trauma admitted to emergency service]. Turkiye Klinikleri J Med Sci 2000;20(3):123-31.
27. Dietrich WD, Alonso O, Busto R, Prado R, Zhao W, Dewanjee MK, et al. Posttraumatic cerebral ischemia after fluid percussion brain injury: an autoradiographic and histopathological study in rats. Neurosurgery 1998;43(3):585-93.
28. Talwalkar SS, Bon Homme M, Miller JJ, Elin RJ. Ischemia modified albumin, a marker of acute ischemic events: a pilot study. Ann Clin Lab Sci 2008;38(2):132-7.