*Turkish Neurosurgery, 2004, Vol: 14, No: 3-4, 71-75*

# Proton Magnetic Resonance Spectroscopy of the Hippocampus After Severe Traumatic Brain Injury

## Şiddetli Beyin Travmasından Sonra Hipokampusun Proton Manyetik Rezonans Spektroskopisi

**Fatma ÖZLEN**
**Galip Zihni SANUS**
**Halil AK**
**Mustafa UZAN**

Department of Neurosurgery,
Cerrahpaşa Medical Faculty,
İstanbul University, İstanbul, Turkey

### ABSTRACT

**OBJECTIVE:** Atrophic changes in the hippocampus that maybe seen during the late stage of traumatic brain injury (TBI) has been found to be highly related to the cognitive function. Previous studies detected by MR morphometric analysis have described atrophic changes in the hippocampus in patients, particularly with no hippocampal lesion in MRI. Use of different imaging parameters and quantification techniques may result in different results and we therefore used MRS to investigate the neuronal loss of the hippocampus. The goal of this study was to show the neuronal loss of hippocampus at the late stage of trauma, with hippocampal MRS in patients with a normal posttraumatic hippocampal MRI.

**METHODS:** Proton magnetic resonance spectroscopy (MRS) was used to evaluate the hippocampus of 13 severe TBI patients. Five of the patients had already regained consciousness while eight were still in the persistent vegetative state (PVS). There were no hippocampal lesions seen in the MRI of the subjects. The patients were divided into two groups according to time interval after the TBI. Eight patients had been evaluated earlier than 12 months (Group A) and five had been evaluated after the first year (Group B). NAA/Cr rates were measured bilaterally by performing long Te single voxel proton spectroscopy of the hippocampus. The results were compared with those of five healthy volunteers (Group C).

**RESULTS:** Comparing Group B and C, there was a significant decrease in the NAA/Cr ratio ($p<0.01$) for both the right and left hippocampus. However, when Groups A and C were compared, the results were only statistically significant for the left side ($p<0.01$). There was no relation between the NN/Cr ratio and the Glasgow Outcome Scale (GOS).

**CONCLUSION:** MRS can be an indicator of neuronal loss in the hippocampus after severe TBI. The significance of the results in the late posttraumatic period is an indicator of hippocampal atrophy.

**KEY WORDS:** Traumatic brain injury, magnetic resonance spectroscopy, hippocampus

### ÖZ

**AMAÇ:** Travmatik beyin hasarının geç dönemlerinde hipokampusta ortaya çıkabilen atrofik değişikliklerin kognitif fonksiyonlar üzerine olan etkisi bilinmektedir. Morfometrik MR analizleri ile yapılan daha önceki çalışmalarda, özellikle MR incelemelerinde hipokampal lezyon görülmeyen hastalarda atrofik değişiklikler saptandığı belirlenmiştir. Farklı görüntüleme parametreleri ve nicel teknikler volümetrik çalışmalarda farklı sonuçlar ortaya çıkarabilmektedir ve bu nedenle hipokampusun nöronal kaybının ortaya konması amacıyla MRS kullanılmıştır. Bu çalışmanın amacı, postravmatik hipokampal MR incelemesi normal bulunan hastalarda, travmanın geç döneminde hipokampustaki nöronal kaybı hipokampal MRS kullanarak göstermekti.

**YÖNTEM:** Ciddi travmatik beyin hasarlı 13 hastada hipokampusu incelemek amacıyla proton magnetik rezonans spektroskopi yapıldı. Hastaların 5'i uyanıklık durumuna dönmüş; 8'i ise halen PVS durumunda bulunuyordu. Hastaların MR incelemelerinde görülen herhangi bir hipokampal lezyon yoktu. Travmatik beyin hasarının oluşmasından itibaren geçen zaman intervaline göre hastalar iki gruba ayrıldı. Sekiz hasta 12 aydan daha erken değerlendirildi (Grup A) ve 5 hasta birinci yıldan sonra değerlendirildi (Grup B). Hipokampusun uzun Te single voxel proton spektroskopisi yapılarak NAA/Cr oranları bilateral ölçüldü. Elde edilen sonuçlar beş sağlıklı gönüllüden oluşan kontrol grubu ile karşılaştırıldı (Grup C).

**BULGULAR:** Grup B ve C karşılaştırıldığında, sağ ve sol her iki hipokampusta da NAA/Cr oranında belirgin bir azalma vardı. Buna karşın, grup A ve C karşılaştırıldığında, sonuçlar sadece sol tarafta istatistiksel olarak anlamlı idi. NAA/Cr oranı ve GOS arasında ise herhangi bir ilişki saptanmadı.

**SONUÇ:** Sonuçlar, MRS'in ciddi travmatik beyin hasarı sonrasında hipokampusta nöronal kaybı değerlendirmek amacıyla kullanılabileceğini ve özellikle geç posttravmatik periyotta hipokampal atrofiyi göstermekte değerli olduğunu desteklemektedir.

**ANAHTAR SÖZCÜKLER:** Travmatik beyin hasarı, manyetik rezonans spektroskopi, hipokampus

Received : 19.12.2003
Accepted : 10.06.2004

Correspondence Address
**Fatma ÖZLEN**
P.K.: 6 Cerrahpaşa,
34099, İstanbul,Turkey
Phone : +90 212 414 30 00-22112
Fax : +90 212 414 3427
E-mail : fatmaozlen@yahoo.com

## INTRODUCTION

The hippocampus and amygdala are part of the limbic system and play a very important role in memory and emotion (15). Because of the crucial role the hippocampus plays in memory and cognition (1, 13), the need to study hippocampal changes associated with a broad spectrum of disorders beyond dementia and epilepsy is apparent (1, 13, 17). Hippocampal atrophy as a consequence of TBI has been documented in animal models (1, 10). Traumatic brain injury produces hippocampal atrophy and temporal lobe enlargement. However, the mechanism by which this occurs is unknown (5).

Protons (1H) have been traditionally used for MR spectroscopy because of their high natural abundance in organic structures and high nuclear magnetic sensitivity compared with any other magnetic nuclei (3, 12). N-Acetyl Aspartate (NAA) is accepted as a neuronal marker and, as such, its concentration will decrease with many insults to the brain (3, 14). The exact role of NAA in the brain is not known. Glutamate and N-acetyl-aspartyl-glutamate are colocalized with NAA in neurons. Breakdown of N-acetyl-aspartyl-glutamate releases both NAA and glutamate, and subsequent breakdown of NAA leads to aspartate. These compounds are excitatory amino acids and increased with ischemia (3).

MR spectroscopy can be used to detect the presence of cerebral metabolites and to measure their amount noninvasively (7, 11). *In vivo* proton MR spectroscopy is now being applied in many clinical fields owing to its easy integration into clinical MR systems and the high magnetization and natural abundance of protons (7, 11, 18). In addition to the structural information obtained by MR imaging, MR spectroscopy can provide spatially encoded biochemical information (11).

## MATERIAL AND METHOD

*Patient population:*

Thirteen patients who had been admitted to the intensive care unit after severe TBI were evaluated retrospectively (Table I). There were nine males and four females and their age ranged from 12 to 65 years (mean 34.8). Data including age, sex, nature of

| No. | Age (y/Sex) | Etiology | GCS | Trauma-MRS interval (month) | MRI | NAA/Cr L-Hippoc. | NAA/Cr R-Hippoc. | GOS |
|---|---|---|---|---|---|---|---|---|
| 1 | 65/M | MVA | 7 | 4 | ICH, DAI | 1,47 | 1,07 | SD |
| 2 | 40/M | MVA | 4 | 36 | EDH, Art (R hippocampus) | 1,18 | 0,94 | MD |
| 3 | 18/M | MVA | 6 | 40 | DAI | 1,63 | 1,48 | MD |
| 4 | 20/M | MVA | 6 | 44 | C (multiple), DAI, Atr (diffuse) | 1,18 | 1,35 | SD |
| 5 | 26/F | Assault | 6 | 16 | DAI | 0,68 | 1,36 | SD |
| 6 | 28/M | MVA | 4 | 5 | DAI, dp. Sk.fx | 1,89 | 1,95 | VS |
| 7 | 42/F | MVA | 4 | 4 | ICH | 1,07 | 2,49 | VS |
| 8 | 32/M | MVA | 6 | 44 | C (multiple), DAI, Atr (diffuse) | 1,26 | 1,65 | PVS |
| 9 | 56/F | Assault | 5 | 3 | C (multiple), DAI, | 1,59 | 1,79 | VS |
| 10 | 56/F | Assault | 5 | 2 | DAI | 1,28 | 0,96 | VS |
| 11 | 40/M | MVA | 5 | 6 | C (multiple), DAI, Atr (diffuse) | 0,81 | 0,85 | PVS |
| 12 | 12/M | MVA | 5 | 3 | DAI | 1,35 | 1,50 | VS |
| 13 | 18/M | Fall | 5 | 3 | DAI | 1,36 | 1,52 | VS |

**Table I:** Demographics, Clinical and Radiological Data and NAA/Cr ratios in the patients with traumatic brain injury who underwent proton MR spectroscopy GCS = Glasgow Coma Score; GOS = Glasgow Outcome Scale; NAA = N-acetylaspartate; CR = Creatine; MVA = motor vehicle accident; ICH = intracerebral hemorrhage; DAI = diffuse axonal injury, EDH = epidural hematoma; Atr = atrophy; C = contusion; dp. Sk. fx. = depressed skull fractures; SD = severe disability; MD = moderate disability; VS = vegetative state; PVS = persistent vegetative state

trauma, Glasgow Coma Score (GCS) and neurological status at admission were obtained. The intensive care and clinical charts and follow-up data were collected. All the patients were evaluated by the same senior author. The length of the post-traumatic period, and changes in the neurological status according to the Glasgow Outcome Scale (GOS) were recorded for each patient and the length of recovery period for those who regained their awareness was determined.

Five patients had already regained their awareness while eight were still in the vegetative state.

*Magnetic Resonance Imaging (MRI) and Magnetic Resonance Spectroscopy (MRS) procedures:*

All patients underwent MRI and the same MRI and MRS protocols were used for all the patients. MRI scans were performed with the 1.5 T Signa (GE, Milwaukee) using version 5.3 software and a clinical protocol which included spin-echo T1-weighted images and fast spin-echo T2-weighted images in two different orthogonal planes at the minimum. All single voxel proton spectroscopy studies were performed with the 1.5 T clinical scanner using the PROBE/SV software (GE, Milwaukee) which permitted automated shimming, water suppression, and data processing. T1-weighted sagittal images were used to locate the voxels in the hippocampi (Figure 1A and 1B). Average voxel volume was approximately 4x2x1.5cm$^3$. Long TE spectra were obtained in all patients using PRESS (TR:1500ms, TE:270ms, 128 acquisitions). After immediate automatic processing of the raw data, we evaluated spectra qualitatively. The spectra were quantified by peak measurements. Ratios of metabolites relative to creatine (Cr) were calculated and compared with the values obtained from controls in the same age range.

*Statistical analysis:*

The metabolite ratios in control and patient groups were compared with ANOVA. Probability values of less than 0.05 were considered significant, and values less than 0.001 were considered highly significant.

## RESULTS

There were no hippocampal lesions seen in the MRI of the subjects. The patients were divided into two groups according to time interval from TBI. Eight patients had been evaluated earlier than 12 months after TBI (Group A) and five had been evaluated after the first year (Group B) by performing long Te, single voxel proton spectroscopy of the hippocampus. NAA/Cr rates were measured bilaterally. The results were compared with those of five healthy volunteers (Group C). Comparing Group B and C, there was a significant decrease in the NAA/Cr ratio ($p<0.01$) for both the right and left hippocampi (Figure 2A and 2B). However, when Groups A and C were compared, the results were

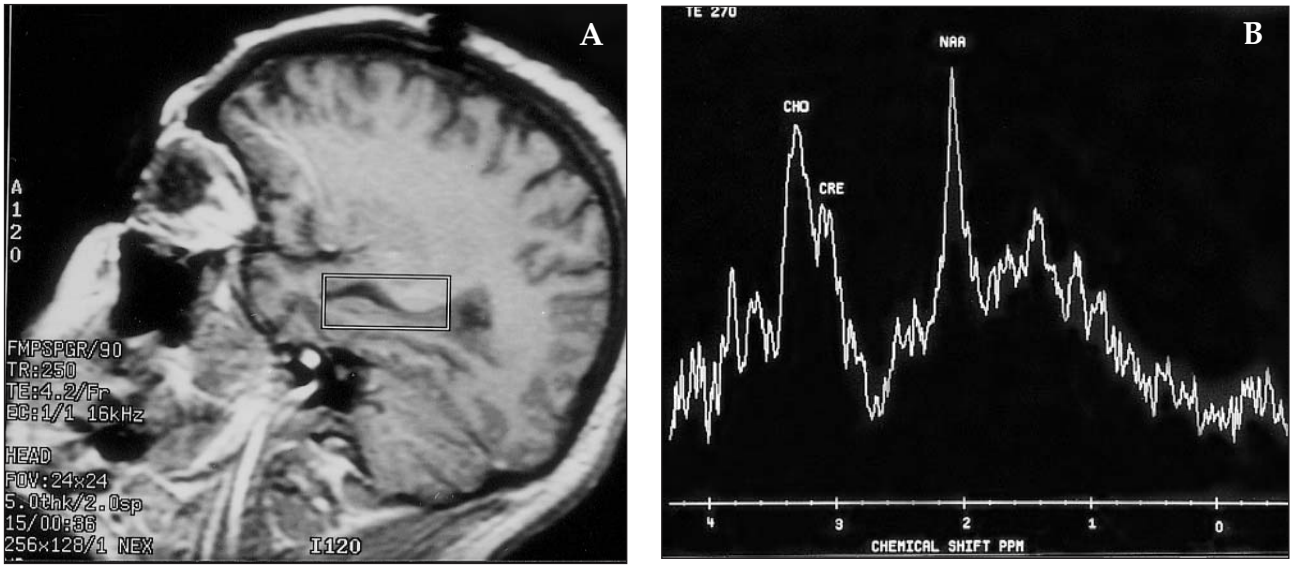


**Figure 1A:** Sagittal MR T1-weighted image shows the position of the voxel with respect to the hippocampal head and body
**B:** Single – volume proton MR spectroscopy shows patterns with decreased NAA and elevated Cho and Cr.

only statistically significant only for the left side ($p<0.01$) (Figure 2B). There was no relation between the NAA/Cr ratio and GOS.

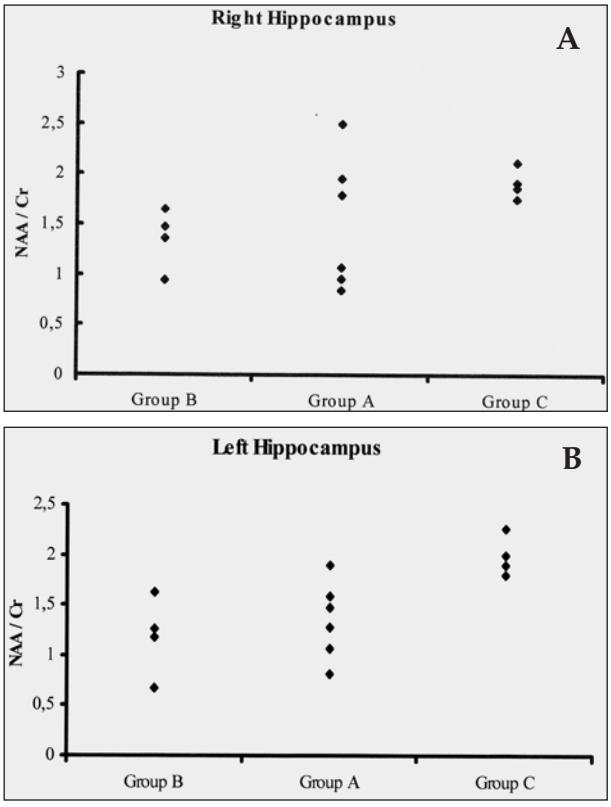


**Figure 2:** Comparing Group B and C, there was a significant decrease in the NAA/Cr ratio ($p<0.01$) for both the right (A), and left (B) hippocampus. The results were only statistically significant in the left side (B) when Groups A and C were compared.

## DISCUSSION

Since there has been a significant decrease in the mortality of the patients with severe TBI, the prediction of the long-term cognitive outcome has attained great importance. Hippocampal atrophy produced by severe TBI has been documented in both human and animal studies. As the hippocampus and amygdala are part of the limbic system and play a very important role in memory and cognition, neural destruction of the hippocampi may result in cognitive deterioration. Using segmentation and volume analyses, Bigler et al (1) have shown that TBI results in atrophic changes in the hippocampus and a decrease in hippocampal volume with an increase in temporal horn volume. In the subacute phase of recovery, the temporal horns were highly predictive of intellectual function and the hippocampi were highly related to verbal memory function (1). As a reliable tool of quantification, volumetric analysis may provide only indirect information on the quantity of the structure. On the other hand, use of volumetric analysis requires an expert in neuroanatomy to define the neuroanatomic boundaries of the hippocampus, and also to manually separate the hippocampal head from the overlying amygdala (5). MRS is a noninvasive method which is able to quantify the metabolic state of the brain and its changes within time and can add invaluable metabolic information to the results of an MR imaging study when incorporated into the routine MR examination (11). The metabolic state of the brain can be estimated with this method by measuring neurochemicals such as NAA, Cr, and Cho.

NAA, a compound synthesized exclusively in neurons, is known to be the most important detectable metabolite in MRS of the brain and is accepted as a neural marker (3, 14). NAA is found in the neural cell bodies in the gray matter, while it is mostly in the axons in the white matter (12). Previous studies have demonstrated that the reduction in NAA intensity in the hippocampus of seizure patients reflects the neural loss or damage (4, 6, 8, 9, 16, 18).

MRS might be a helpful and noninvasive test with which to lateralize the abnormal hippocampus of seizure patients (18). In our study, we used MRS to evaluate the hippocampus of patients with TBI to determine whether there was any neural destruction. Comparing the cases that were evaluated after the first year with healthy volunteers, we found a significant decrease in NAA/Cr ratio for both right and left hippocampus. However, when the cases that were evaluated earlier than 12 months were compared with the same healthy subjects, we found that the only significant result was for the left side. These findings are consistent with previous studies in which a particular time frame following the injury (2.5 to 7 months) was mentioned for predicting outcome (1, 2). MRS appears to be an effective and reliable method for evaluating the hippocampus in the late phase of TBI at this point. Another finding of this study has been that no relation was found between the NAA/Cr ratio and the Glasgow Outcome Scale. We think that the GOS is probably more related to the primary damage of TBI. In conclusion, the hippocampus needs to be evaluated

in patients with TBI as it plays an important role in cognitive capability. When used after one year following the injury, MRS provides an objective metabolic assessment of the neural state of the hippocampus. More detailed analysis of the hippocampus using both morphometric and metabolic techniques may provide greater specificity.

**ACKNOWLEDGEMENT**

The authors thank Alp Dinçer, M.D. from Radyomar Radiology Center for the MR spectroscopy analysis.

**REFERENCES**

1. Bigler ED, Blatter DD, Anderson CV, Johnson SC, Gale SD, Hopkins RO, Burnett B: Hippocampal volume in normal aging and traumatic brain injury. Am J Neuroradiol 18; 11-23, 1997
2. Blatter DD, Bigler ED, Gale SD, Johnson SC, Anderson CV, Burnett BM, Ryser D, Macnamara SE, Bailey BJ MR: -based brain and cerebrospinal fluid measurement after traumatic brain injury: correlation with neuropsychological outcome. Am J Neuroradiol 18; 1-10, 1997
3. Castillo M, Kwock L, Mukherji SK: Clinical applications of proton MR spectroscopy. Am J Neuroradiol 17; 1-15, 1996
4. Cendes F, Andermann F, Preul MC, Arnold DL: Lateralization of temporal lobe epilepsy based on regional metabolic abnormalities in proton magnetic resonance spectroscopic images. Ann Neurol 35; 211-216, 1994
5. Clifford RJ: Medial temporal lobe volumetrics in traumatic brain injury. Am J Neuroradiol 18; 25-28, 1997
6. Connelly A, Jackson GD, Duncan JS, King MD, Gadian DG: Magnetic resonance spectroscopy in temporal lobe epilepsy. Neurology 44; 1411-1417, 1994
7. Cousins JP: Clinical MR spectroscopy: fundamentals, current applications, and future potential. Am J Roentgenol 164; 1337-1347, 1995
8. Ende GR, Laxer KD, Knowlton RC, Atson GB, Schuff N, Fein G, Weiner MW: Temporal lobe epilepsy: bilateral hippocampal metabolic changes revealed at proton MR spectroscopic imaging. Radiology 202; 809-817, 1997
9. Gadian DG, Connelly A, Duncan JS, Cross JH, Kirkham FJ, Johnson CL, Vargha K, Nevile BG, Jackson GD: 1H Magnetic resonance spectroscopy in the investigation of intractable epilepsy. Acta Neurol Scand 152 (suppl); 116-121, 1994
10. Hicks RR, Smith DH, Lowenstein DH, Saint-Marie R, Mcintosh TK: Mild experimental brain injury in the rat induces cognitive deficits associated with regional neuronal loss in the hippocampus. J Neurotrauma 10; 405-414, 1993
11. Hsu YY, Chang C, Chang CN, Chu NS, Lim KE, Hsu JC Proton MR: spectroscopy in patients with complex partial seizures: single-voxel spectroscopy versus chemical-shift imaging. Am J Neuroradiol 20; 643-651, 1999
12. Lenkinski RE, Schnall MD MR: spectroscopy and the biochemical basis of neurological disease. In: Atlas SW, ed. Magnetic Resonance Imaging of the Brain and Spine. New York: Raven, 1991:1099-1121
13. McCarthy G: Functional neuroimaging of memory. NeuroScientist 1; 155-163, 1995
14. Miller BL: A review of chemical issues in 1H NMR spectroscopy: N-acetyl-L-aspartate, creatine, and choline. NMR Biomed 4; 47-52, 1991
15. Otsuka H, Harada M, Mori K, Hisaoka S, Nishitani H: Brain metabolites in the hippocampus- amygdala region and cerebellum in autism: an 1H-MR spectroscopy study. Neuroradiology 41; 517-519, 1999
16. Peeling J, Sutherland G 1H Magnetic resonance spectroscopy of extracts of human epileptic neocortex and hippocampus. Neurology 43; 589-594, 1993
17. Soininen HS, Partanen K, Pitkanen A, Vainino P, Hanninen T, Hallikainen M, Koivisto K, Riekkinen PJ: Volumetric MRI analysis of the amygdala and the hippocampus in subjects with age- associated memory impairment: correlation to visual and verbal memory. Neurology 44; 1660-1668, 1994
18. Thompson JE, Castillo M, Kwock L, Walters B, Beach R: Usefulness of proton MR spectroscopy in the evaluation of temporal lobe epilepsy. AJR 170; 771-776, 1998