TJTES
TURKISH JOURNAL OF TRAUMA
& EMERGENCY SURGERY

Ulusal Travma Dergisi (2002) 8: 74 - 77

# HAYVAN KAFA TRAVMASI MODELİNDE DOKU VE SERUM SİALİK ASİT SEVİYELERİ

# TISSUE AND SERUM SIALIC ACID LEVELS IN AN ANIMAL HEAD INJURY MODEL

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*ÖZET*

*Amaç: Bazı santral sinir sistemi hastalıklarını da içeren değişik hastalıklarda serum sialik asit düzeyinde belirgin yükselme saptanır. Kafa travmasında serumda ve beyin dokusunda sialik asit düzeyini araştıran bir çalışma yoktur. Bu nedenle, kafa travmasında serumda ve beyin dokusunda sialik asit düzeylerini değerlendiren bir çalışma planladık.*

*Gereç ve Yöntem: Sıçanlarda yaygın beyin hasarı oluşturmak amacıyla Marmarou'nun darbe-akselerasyon modeli kullanıldı. Sıçanlar üç gruba ayrıldı. 450 gr'lık bir ağırlık deneklerin kafasına I. grupta 1 m yükseklikten, II. grupta 2 m yükseklikten düşürüldü. III. grup kontrol grubunu oluşturdu. Travmadan sonra hem serumda, hem beyin dokusu süpernatantlarında sialik asit düzeyi ölçüldü.*

*Bulgular: Serum sialik asit düzeyi travmanın şiddetine ve travma sonrası geçen süreye bağlı olarak azalma eğilimi gösterdi. Buna karşın beyin dokusundaki doku sialik asit düzeyinde anlamlı bir değişiklik olmadı.*

*Sonuç: Serum sialik asit düzeyi yaygın beyin hasarının derecesini gösteren bir marker olarak kullanılabilir.*

*Anahtar kelimeler: Sialik asit, yaygın beyin hasarı.*

*ABSTRACT*

*Background: Significant elevations of serum sialic acid level have been documented in various diseases including in a variety of central nervous system disorders. But, in head injury, there is no any study on the serum and brain tissue sialic acid levels. So, we planned an experimental study to evaluate serum and brain tissue sialic acid levels in head injury.*

*Methods: Marmarou's impact-acceleration model was used in rats to produce diffuse brain injury. Rats were divided into equal three groups. In Group I, 450 g weight was fell from 1 m height to heads of subjects, and from 2 m in Group II. Group III was control group. Sialic acid levels were measured in both sera and brain tissue supernatants after trauma.*

*Results: It was observed that serum sialic acid level was decreased according to the severity and period of trauma increased; and there was no change in brain tissue sialic acid levels.*

*Conclusion: Serum sialic acid level might be used as a marker to show the degree of diffuse brain injury.*

*Key Words: sialic acid, diffuse brain injury*

## INTRODUCTION

Sialic acid (SA) is a general name given to a family of compounds derived from the 9-carbon sugar neuraminic acid (1). SAs play a central role in the biomedical functioning of human (2). They are bound to the non-reducing end of carbohydrate chains of glycoproteins and glycolipids (3) and many of them represent important components of cell membranes. They carry a negative charge at physiological pH, affecting the conformation of glycoconjugates, and play a crucial role in the properties of cell surfaces: acting as recognition determinants in host-pathogen interactions and cell-cell interactions, affording protection from membrane proteolysis, and being required for activation of receptors by hormones (1). SAs form a negative charge on cell surfaces and prevent aggregation (4). In addition, they are not only structural components of membranes, but also of nuclei and cell organelles (5).

Cerebral tissue is also too rich from SAs. Gangliosides, found on outer leaflet of lipid layer of the plasma membrane particularly in brain, are sialylated glycosphingolipids (6). Polysialic acid (PSA) is a carbohydrate composed of SA residues and implicated in many morphogenic events of the neural cells (7). Both gangliosides and polysialic acid have important functions on neural tissue (6-8).

Sas are found in small amounts in many body fluids, such as serum, urine, saliva, semen, cerebrospinal fluid (CSF) and pleural effusion (1). Numerous recent studies have addressed themselves to the quantification of serum or pleura sialic acid in various disease state. Significant elevations of total circulating SA have been documented in

renal diseases, in diabetes, in Behcet's disease, in bacterial infections, in inflammatory diseases such as Chron, pseuriasis, arthritis, and also in a variety of central nervous system disorders (1,9,10). But, in head trauma, there are no any experimental or clinical studies about the serum and brain tissue SA levels. So, in this study we performed an animal study evaluating the association between serum and brain tissue SA levels in diffuse brain injury and the effects of severity of the trauma on serum and tissue SA levels.

## MATERIALS AND METHODS

In this study, 24 Spraque-dawley male rats were used. Average total body weight of the subjects was 254,8±25,4 gr. To produce diffuse brain injury, Marmarou's impact-acceleration model was used (11). Rats were divided into equal three groups. In Group I, 450 g weight was fell from 1 m height to heads of subjects, and from 2 m in Group II. Group III was control group.

In Groups I and II, 30 mg/kg thiopental was given to the subjects intraperitoneally. After endotracheal intubation, scalp was incised on midline and a stainless steel disc in 1 cm radius was tightly stuck to cranium on midline between coronary and lambdoid sutures. A 450 g weight was fell to the disc through a plexiglass tube from 1 m in Group I and 2 m in Group II. Immediately after trauma, the subjects were followed for a few minutes and were ventilated mechanically by a respirator.

Blood samples were obtained 30 and 60 minutes after trauma by method of tail tip cutting in Group I and II, and centrifuged on 3000 rpm for 5 minutes. In addition, blood samples were taken out in Control Group. Sera were kept on -20°C.

The subjects were decapitated 60 minutes after trauma and all of the brain tissues were taken out. Brain tissues were washed out with serum physiologic and homogenated in 0,05 M (pH=7,4) phosphate buffer on +4°C, thus, a 20% tissue homogenates were prepared. They were centrifuged at 20000 rpm for 10 minutes. Supernatants were also kept on -20°C. The subjects of Control Group were also decapitated after blood samples were taken out and supernatants were prepared in the same manner.

SA levels were determined by Warren's thiobarbituric acid assay in both serum and tissue supernatants (12). Values were determined as mg/dl in serum and mg/gr moist tissue in neural tissue.

**Statistical Analysis:**

The results were compared between the groups by two-tailed homoscedastic or heteroscedastic t-test according to F test results; and the serum and tissue SA levels were compared in the same group by correlation. It was accepted as significantly if $p<0,05$ is accepted as significant difference.

## RESULTS

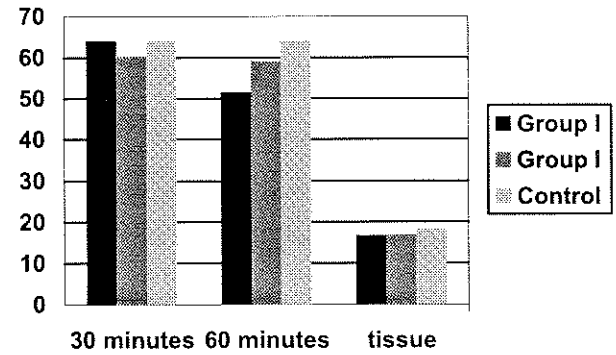
Average serum and tissue SA levels of the groups were shown in Table I and Graphic I. In both trauma groups, the serum SA levels showed an inclination to decrease according to control values when the severity and period of trauma increase (Table I). But only significant difference was observed between serum SA levels of the Group I-60 minutes after trauma and Control Group ($p=0,02$); the difference between serum SA levels of Group II-60 minutes after trauma and Control Group was not statistically significant ($p=0,054$).

There were no significant differences between tissue SA levels of trauma groups and control group; and also there were no significant correlation between serum and tissue SA levels in the same group for all groups.

Table 1: Average serum and tissue sialic acid levels of the groups.

| (Average±SD) | Serum-30 min. (mg/dl) | Serum-60 min. (mg/dl) | Tissue (mg/gr moist tissue) |
|---|---|---|---|
| Group I (1 m) | 65,28±10,98 | 55,68±6,46 | 16,17±1,88 |
| Group II (2 m) | 60,16±7,84 | 58,92±4,03 | 16,91±0,92 |
| Control Group | 65,5±5,81 | | 18,24±3,16 |

Graphic 1: Diagram of average serum and tissue sialic acid levels of the groups.




## DISCUSSION

SAs are bound to the non-reducing end of carbohydrate chains of glycoproteins and glycolipids (3). Decreases of SA amounts of proteins due to various conditions may be caused proteolysis and tissue damage (13). Gorog has stated that carbohydrate chains of proteins

protect those proteins from proteolysis (13). In various pathological conditions, elevated serum SA levels as the result of increased metabolism of glyco-proteins and glycolipids are observed (2). The relationship between abnormalities in serum SA concentrations and cellular SA is unknown; but the significant association between plasma SA levels and platelet SA suggests that increasing vascular damage is associated with increasing turnover and shedding of structural glycoproteins into circulation (14).

However, increased serum SA concentrations have been reported during various diseases, the apparent non-specificity of SA in such disease limit the potential usefulness of SA levels. In addition, some non-pathological conditions, such as ageing, pregnancy and smoking may cause changes in serum SA concentrations (2). The absolute increases in SA levels are also rather small; this condition further limits the clinical potential of SA as a marker (2). The vast majority of serum SA is covalenty bound to glycoproteins. A wide range of functional circulating proteins are sialylated. The lipids termed gangliosides also bind the SA in serum. So, free SA represents a minute fraction of the total in serum (2).

Cerebral tissue is highly rich from SAs. Gangliosides, found on outer leaflet of lipid layer of the plasma membrane particularly in brain, are sialylated glycosphingolipids (6). They have been suggested to play a prominent role in both normal and abnormal developmental processes. In addition, several lines of convergent evidence have indicated that gangliosides exert pronounced trophic effects following damage to peripheral and central nerves (8). They reduce oedema formation, restore glucose metabolism, and increase cerebral blood flow after focal ischemia in the rat brain (16,17). To apply gangliosides to neurones after trauma in an experimental study has prevented activity decrease of some important enzymes such as $Na^+K^+$ATPase (18).

Another sialylated substance found in brain tissue, polysialic acid (PSA), is a carbohydrate composed of SA residues (7). It is mainly attached to the neural cell adhesion molecule (N-CAM) and implicated in many morphogenic events of the neural cells by modulating the adhesive property of N-CAM (7,19). Polysialic acid and N-CAM are closely related to axonal pathfinding, targeting, and synaptic activitles (20).

It is expected that serum SA level may be increased in diffuse brain injury because of tissue damage, destruction of plasma membrane and blood-brain barrier in diffuse brain injury. In addition, brain tissue is highly rich in SAs. Other conditions, in which tissue damage occurs, such as surgical trauma, it has been observed that serum SA is increased. In the patients with thyroidal (21), prostate and bladder cancer (22), serum SA levels have increased 24 hours after surgery and it has been stated that this condition is due to surgical trauma. However in the presented study, it was observed that serum SA level was decreased as the severity and period of trauma increased; and there is no change in tissue SA levels. Perhaps, the decrease of serum SA in brain injury in time may be explained by systemic results of diffuse brain injury. So, some new studies evaluating serum and tissue SA levels together with other systemic effects of head trauma may be planned to explain of this decrease.

It has been reported that serum SA level may be used as a marker showing the risk of cardiovascular mortality (23,24), or the grading, improvement or deterioration of the disease in cancer patients (10,25-28). In this study, we found an inclination to decrease in serum SA levels as the severity and period of diffuse brain injury increased. So, it was thought that serum SA level might be used as a marker to determine the degree of diffuse brain injury.

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