

# Cerebral Tissue Sialic Acid Levels in Experimental Brain Edema

## Deneysel Beyin Ödeminde Serebral Doku Sialik Asit Seviyeleri

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**Abstract: Objective:** The aim of this study was to examine sialic acid levels in brain tissue with vasogenic edema due to experimental focal freeze injury.

**Methods:** Thirty-seven male Wistar-Albino rats were divided into an edema group (n=30) and a control group (n=7). Animals in the edema group were subjected to focal freeze injury in the left parietal dura. The control rats were not subjected to freeze injury or any other manipulation or drug. All the rats were decapitated 24 hours after the injury in the edema group, and brain tissue sialic acid levels were determined using Warren's thiobarbituric acid assay.

**Results:** The rats in the edema group had significantly higher cerebral tissue sialic acid levels than the rats in the control group.

**Conclusion:** There is still lack of consensus in the literature, but these study results indicate that high sialic acid levels in cerebral tissue with vasogenic edema may reflect the severity of brain injury, and may be a useful marker of severity of edema in the clinical setting.

**Key Words:** Blood brain barrier, brain edema, sialic acid

**Özet: Amaç:** Bu çalışmanın amacı fokal soğuk yaralanmayı takiben oluşturulan vazojenik beyin ödeminde serebral doku sialik asit seviyesinin ölçülmesidir.

**Yöntem:** Bu deneysel çalışmada, sırasıyla 30 ve 7 adet erkek Wistar-Albino sıçanlarından oluşan ödem ve kontrol olmak üzere 2 grup oluşturulmuştur. Kontrol grubundaki hayvanlar fokal soğuk yaralanmaya tabi tutulmuş ve 24 saat sonra dekapitasyon uygulanmıştır. Kontrol grubunu oluşturan hayvanlar da aynı şekilde dekapite edilmiştir. Beyin dokusu sialik asit seviyeleri Warren tiobarbitürik asit yöntemiyle değerlendirilmiştir.

**Bulgular:** Hasar oluşturulan beyin dokusundaki sialik asit seviyelerinin kontrol grubuna göre yüksek olduğu görülmüştür.

**Sonuç:** Literatürde henüz ortak bir görüş bildirilmemesine rağmen, bu çalışmanın sonucunda ödem takiben ortaya çıkan yüksek sialik asit, klinikte hasar derecesini saptamada bir belirteç olarak kullanılabilir.

**Anahtar Kelimeler:** Beyin ödemi, kan-beyin bariyeri, sialik asit

## INTRODUCTION

Cerebral edema (CE) is defined as abnormal accumulation of water in the intra and/or extracellular spaces of brain tissue. CE is classified as four different types: vasogenic, cytotoxic, osmotic and hydrostatic. Of these, only vasogenic edema is caused by breakdown of the blood-brain-barrier (BBB). This form of CE is primarily extracellular, and usually occurs in response to trauma, primary or metastatic brain tumors, focal inflammation, or in the later stages of cerebral ischemia (1,5).

Sialic acids are Nitrogen and Oxygen acetylated derivatives of neuraminic acid. These acids are components of many complex carbohydrates that are comprised of glycoproteins and glycolipids. In the brain, sialic acids are mainly found in gangliosides, which are galactose-containing cerebrosides that are important in neuron regeneration after primary or secondary injury (3).

The aim of this experimental study was to compare sialic acid levels in normal rat brain to levels in rat brain tissue with vasogenic brain edema caused by focal freeze injury. To the best of our knowledge, this is the first investigation to have examined cerebral sialic acid levels in relation to traumatic brain edema.

## MATERIALS AND METHODS

This study was approved by the Animal Care Committee of the University of Istanbul. Thirty-seven male Wistar-Albino rats weighing 200-230 g and aged 3-4 months were divided into an edema group (n=30) and a control group (n=7). All animals were given free access to water and food. The animals in the edema group were anesthetized with an intraperitoneal injection of ketamine hydrochloride (50 mg/kg).

Craniectomy and the freeze injury procedure were performed according to the method of Tominaga and Ohnishi (15). Each anesthetized rat was secured to a smooth surface in prone position. A vertical skin incision was made in the midline of the scalp, and the sagittal and the left coronal sutures were identified. The periosteum covering

the left parietal region was dissected away from the midline, and the temporal muscle was dissected to expose its attachment to the temporal bone. Using a dental drill and irrigating constantly with isotonic solution to keep the tissues cool, a small burr hole was drilled near the center of the parietal bone. This hole was enlarged using a mini-curved hemostat, and care was taken to ensure the dura remained intact. A piece of cranium measuring approximately 10x15 mm was removed, starting at the left mid-sagittal plane and extending to the temporoparietal region. Bonewax® (Ethicon) and Surgicel® (Ethicon) were used to stop bleeding as required and extreme care was taken to avoid damaging the sagittal sinus. Once the left parietal dura was exposed, a 4x10-mm metal probe immersed in liquid nitrogen at -70 °C was used to produce a focal freeze injury. The probe was kept in contact with the exposed dura covering the left parietal lobe for 45 seconds, and the site was then closed with single-layer suturing.

The control animals were subjected to no procedures, and received no anesthesia or other drugs. Twenty-four hours after cold injury produced in the edema group, all animals in both groups were decapitated. Each rat's brain was removed and brain tissue sialic acid levels were determined using Warren's thiobarbituric acid assay (16).

## RESULTS

The respective mean brain-tissue sialic acid levels in the edema and control groups were 0.305±0.0043 and 0.240±0.041 mg/g wet tissue. Figure 1 shows the difference in cerebral tissue sialic acid levels between the edema and control groups.

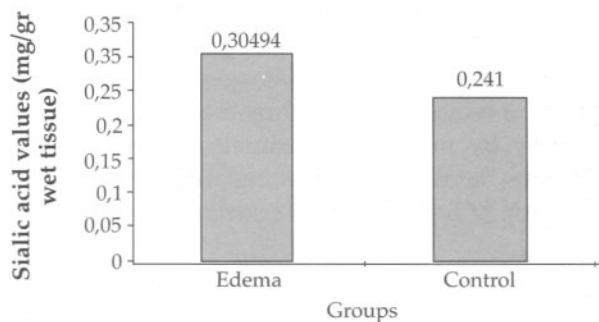


Figure 1: Comparison of the sialic acid levels in the edema and control groups.

## DISCUSSION

Sialic acids are acetyl derivatives of neuraminic acid, and are mainly found in mammalian tissues (8). Majority of sialic acids are bound to the terminal end of oligosaccharides, but they can also bind to the sides of oligosaccharide chains. Large amounts of sialic acids are found in orosomucoid, alpha-1 antitrypsin, haptoglobin, seruloplasmin, fibrinogen, proteins of the complement system, and transferrin in human plasma (11,14). Some of these glycoproteins are known as "acute-phase reactants," which tend to increased in inflammation and injury (12).

The various functions of sialic acids have been studied and reported extensively, and detailed explanation of these is beyond the scope of this paper. However, it is important to note one way that these chemicals affect the cells. Overall, most sialic acids carry a negative charge at physiological pH. Accumulation of negatively charged sialic acid residues on cell membranes strongly influences cell behavior, since the electrostatic repulsion prevents cell aggregation. In addition to effects on cellular interactions, the repulsive forces of sialic acids increase the rigidity of the cell surface and make the surface more fragile (16). Gangliosides are glycosphingolipids that contain endogenous sialic acid and are highly concentrated in the central nervous system (CNS), so play an important role in both normal and abnormal developmental processes. A variety of studies in recent years have exposed the potential use and benefit of gangliosides as treatment for CNS trauma. One study have shown that administration of monosialoganglioside-1 (GM1) significantly improved neurological recovery in the spinal cord injury in rats, even though the drug was not given until approximately 72 hours post-trauma (2). Other reports have documented that gangliosides have obvious trophic effects on damaged peripheral and central nerves (4,8). Also, they mediate functional recovery by minimizing primary and secondary cell loss, and by promoting regeneration or sprouting of injured central neurons (13).

Some of the proposed mechanisms for ganglioside function include modulation of protein kinase C activity (7), release of growth factors (16), and inhibition of the release of excitatory amino

acids (9). In addition, ganglioside treatment seems to prevent decay in the activity of some key enzymes, such as Na-K-ATPase, which is particularly important in cell after trauma (3). More extensive pre-clinical studies of ganglioside treatment in CNS trauma are needed, and further investigation of the possible mechanisms of ganglioside action is also required.

Vasogenic brain edema is the most common form of edema seen in clinical practice. As mentioned above, it most often occurs in response to trauma, tumors, subarachnoid hemorrhage, and in the later stages of cerebral ischemia. It is well known that vasogenic brain edema is associated with BBB impairment and increased levels of lipid peroxidation products (1). It is initiated by breakdown of the BBB, which leads to movement of plasma or plasma filtrates into the extracellular space. The involved region features increased tissue pressure, loss of autoregulation, decreased cerebral blood flow and acidosis. Vasogenic edema will progress as long as there is capillary leakage and a tissue pressure gradient is present. In addition, chemical alterations in the involved tissue may also influence the severity of vasogenic edema (1). Baethmann et al. (1) proposed that glutamate, serotonin, components of the kallikrein-kininogen-kinin (KKK) system and free fatty acids may enter edematous tissue and aggravate the damage. These substances may inflict injury by enhancing local and distal barrier permeability via stimulation of pinocytosis (serotonin, KKK), or by deranging microcirculatory control and thus increasing hydrostatic filtration through the damaged capillaries. Additionally, glutamate and free fatty acids can increase permeability of the cell wall to sodium, leading to cellular swelling.

Perry et al. (10) showed the BBB regulates the expression of a macrophage sialic acid-binding receptor on microglia, which are the resident macrophages of the CNS. Under normal circumstances, microglia do not express the sialic acid-binding receptor named sialoadhesin. The authors' in vitro experiments revealed that expression of sialoadhesin is regulated by exposure to an inducing agent that is present in serum. The investigators used immunocytochemistry to examine the macrophage populations of the nervous system and test whether this inducing

agent in serum also regulates sialoadhesin expression *in vivo*. In addition, they assessed whether plasma proteins influence the phenotype of macrophages of the nervous system. The results showed that CNS injury that impairs the BBB does induce sialoadhesin expression on a proportion of macrophages and microglia within the parenchyma (10).

Sialidase, originally known as neuraminidase, is an enzyme that cleaves terminal sialic acid residues from oligosaccharides, glycoproteins and gangliosides. It has been demonstrated that treatment of nerve cells with sialidase leads to changes their activity (4). One group of researchers showed that injection of this enzyme into presynaptic neurons in squids blocked synaptic transmission (6). The same group observed a significant initial increase in neuron activity when they injected sialidase into squid spinal cord segments. It seems that these changes in activity are related to the degree of sialylation of gangliosides that are bound to calcium ions. Evidence from the studies mentioned above clearly show electronegative charges of sialic acids play an important role in nerve cell activity.

Goettl et al. (4) investigated the effects of aging on responses to thermal and mechanical stimuli in Sprague-Dawley rats. Compared to findings in younger animals, they recorded longer latencies in reaction time when older rats were exposed to the hot plate and found on high-intensity tail flick assays; however, there was no difference between the younger and older groups on the low-intensity tail flick assays. Administration of a type of sialic acid residue (GM1) restored the responses in the hot plate and von Frey hair assays, but had no effect on the slower responses in the tail flick test in aged rats.

In summary, when cerebral tissue becomes damaged, gangliosides play important roles in tissue regeneration. Our results show that sialic acid levels in rat brain are significantly increased in the setting of vasogenic edema. We conclude that brain-tissue sialic acid levels may reflect the severity of cerebral injury, and further clinical and/or experimental studies are required to clarify the importance of sialic acids in traumatized nervous tissue.

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