Relationship Between Serum and Cerebrospinal Fluid Oxidative Stress Index and the Infarct Volume in Patients with Acute Ischemic Stroke

Akut İskemik İnmeli Hastalarda Serum ve Boş Oksidatif Stres İndeksi ile İnfarkt Volümü Arasındaki İlişki

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ABSTRACT Objective: The aim of this study was to investigate the relationship between the oxidative stress index (OSI) values in serum and cerebrospinal fluid (CSF) and the multidetector computed tomography (MCT) infarct volumes, in patients with ischemic stroke. **Material and Methods:** This study included 40 patients (22 females and 18 males) who were hospitalized at the Neurology clinic and who were monitored for ischemic stroke. Infarct volumes of the patients with ischemic stroke were measured with control MCT 48-72 hours later. Blood and CSF samples were collected from all patients after 48-72 hours. Total serum and CSF peroxide concentrations were detected with the FOX-2 method. Total antioxidant capacity in CSF and serum samples was measured with a kit containing ABTS [2-2' -azinobis-(3-ethyl-benzothiazoline-6-sulphonic acid)]. Total peroxide value was divided by total antioxidant capacity value and OSI was determined. The relationship between serum and CSF oxidative stress index and infarction volumes was studied. **Results:** There was a significant corelation between average infarct volumes of all patients and both serum and CSF OSI average values in all patients. **Conclusion:** In conclusion, the finding of a relationship between oxidative stress and infarct volume of the cerebral ischemia might provide new insights into the pathogenesis of ischemic stroke as well as open new therapeutic possibilities. Therefore, we suggest that, OSI may be useful as a marker to determine the progress of the brain tissue damage.

Key Words: Hypoxia-ischemia, brain; oxidative stress; brain ınfarction; cerebrospinal fluid; multidetector computed tomography

ÖZET Amaç: Bu çalışmada, iskemik inme geçiren hastaların serum ve beyin omurilik sıvısı (BOS) incelemelerinde belirlenen oksidatif stres indeksi (OSİ) değerleri ile çok tarayıcılı bilgisayarlı beyin tomografisi (ÇTBBT) incelemesinde tespit edilen infarkt volümü arasında bir ilişki olup olmadığını araştırmayı amaçladık. **Gereç ve Yöntemler:** Bu çalışmaya, Nöroloji kliniğinde yatırılarak takip edilen 22'si kadın 18'i erkek toplam 40 iskemik inmeli hasta alındı. İskemik inmeli hastaların infarkt volümleri 48-72 saat sonra çekilen ÇTBBT ile ölçüldü. Hastaların 48-72 saat sonra alınan kan ve BOS örnekleri biriktirildi. Total peroksit konsantrasyonları hem serum hem de BOS'da FOX-2 metodu ile ölçüldü. Total antioksidan kapasite, hem serum hem de BOS'da ABTS [2-2'-azinobis- (3-ethyl-benzothiazoline-6-sulphonic acid)] ihtiva eden kit yardımıyla ölçüldü. Total peroksitlerin total antioksidan kapasiteye oranı ile OSİ tespit edildi. Serum ve BOS oksidatif stres indeksi ile infarkt volümleri arasındaki ilişki araştırıldı. **Bulgular:** Hastaların ortalama serum ve BOS OSİ değerleri ile ortalama infarkt volümleri arasında belirgin korelasyon mevcuttu. **Sonuç:** Sonuç olarak serebral iskemide infarkt volümü ve oksidatif stres arasındaki ilişkinin gösterilmesi, iskemik inme patogenezinde yeni bir görüş sağladığı gibi, yeni tedavi olanaklarına da zemin hazırlayabilir. Bu nedenle OSİ'nin beyin hasarını tespitte bir araç olarak kullanılabileceğine inanmaktayız.

Anahtar Kelimeler: Hipoksi-iskemi, beyin; oksidatif stres; beyin infarktüsü; serebrospinal sıvı; çok tarayıcılı bilgisayarlı tomografi

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Stroke is among the most important health problems worldwide. It is a neurological condition most commonly associated with focal cerebrovascular diseases with a sudden onset that lasts for more than 24 hours.[1] Stroke occurs after occlusion of the blood vessels supplying blood to the brain or hemorrhage, as a result of changes in the blood vessels and/or in the characteristics of blood circulating through the vessels.[2] It is difficult to predict the cause of stroke before the age of 45. Ischemic strokes account for 80-90% of all stroke cases.[3] Computerized brain tomography (CBT) imaging technique is currently the most effective method in early stages for the patients with acute stroke.[4]

There is oxygen deficiency during ischemia. Endogenous free radical absorbent enzyme and oxidative glutathione rapidly decrease during ischemia. There is an enormous increase in free radicals with the provision of oxygen during reperfusion. Free radicals during ischemia, and especially during reperfusion, greatly contribute to the breakdown of the blood-brain barrier, which in turn generates vasogenic edema and penetration of inflammatory cells into the ischemic region.[5]

The oxidative stress index (OSI) is a new measure determined by the ratio of total peroxide to total antioxidant capacity.[6,7] Oxidative stress can be assessed in all body fluids.[8] The OSI is deterministic for oxidative stress. Publications are available on animal studies demonstrating the relationship between oxidative stress and the infarct volume in stroke. [9]

Oxidative stress is defined as, any disorder in the cellular pro-oxidant/antioxidant equilibrium in favor of pro-oxidants.[10] Oxidation is virtually essential for almost all cells in energy production for vital functions. Oxidative stress is a term used to describe oxidative injury in organs, tissues and cells, which occurs due to reactive oxygen species. This injury can involve internal organs or a specific molecule. Cerebral ischemia initiates complex series of various metabolic events, which result in the development of free oxygen radicals and nitrogen. These free radicals and the related reactive chemical species lead to most of the damage arising in the penumbra region of the infarcts caused by permanent ischemia, and to most of the damage that arise after temporal cerebral ischemia. Cerebral ischemia also leads to the formation of superoxide in addition to synthesis of nitric oxide. Oxidative membrane damage and changes in blood antioxidant capacity have been reported in patients with acute stroke.[11] A relationship has been identified between the clinical outcomes and the production capacity of reactive oxygen species that are compatible with the severity of neurological deficit in patients with stroke.[12] Cell death is most probably due to the nature and severity of the ischemic damage. Various therapeutic strategies have been developed in order to protect individuals from the effects of ischemia and oxidative damage following cerebral ischemia. Despite the ineffective nature of some drugs used in early studies or their unacceptable side effects, some other studies conducted with antioxidant drugs have produced favorable results. Recent results obtained from experimental animal studies may probably lead to the development of advanced pharmacological strategies, which limit brain damage in patients with stroke.[13] The aim of this study was to investigate the relationship between the OSI values in serum and cerebrospinal fluid (CSF) and the multidetector computed tomography (MCT) infarct volumes, in patients with ischemic stroke.

MATERIAL AND METHODS

Patients admitted to the Neurology clinic within 24 h of the onset of acut ischemic stroke symptoms were recruited for this study. The Institutional Ethics Committee approved the study and all participants provided their written informed consent. Inclusion criteria for patients with ischemic stroke were focal neurologic deficit and computed tomography evidence (CT) of infarct. Those with transient ischemic attack (TIA) and reversible ischemic neurological deficit (RIND) were not included in the study. Patients were assessed with medical history, clinical examination, electrocardiography (ECG), hemogram, radiological analysis such as telecardiography, and biochemical analy-

sis. During their first hospitalization and after 48-72 hours all patients were screened with MCT involving multislice technique, and the infarct volumes were determined

Blood samples were collected from all patients after 48-72 hours. They were placed in biochemical tubes and were centrifuged at 3000 rpm for 10 minutes in order to obtain the sera. The obtained sera were kept in a deep freezer at -80ºC until the day of the study. Additionally, CSF samples were obtained 48-72 hours after hospitalization under sterile conditions and without any procedure kept in a deep freezer at -80ºC until the day of the study.

The total antioxidant capacity in both serum and CSF was evaluated using the ferric reducing/antioxidant power (FRAP) developed by Benzie and Strain.[14,15] The results were obtained in μmol Trolox equivalent/liter which was later converted to μmol/l. The total concentrations of peroxide in both serum and CSF were determined using the FOX-2 method.[16]

The OSI was calculated using the following formula:

OSI= [(Total peroxide, μmol/lt/TAOP (Total Antioxidant Power) μmol/lt) ×100].

The total antioxidant capacity in both serum and CSF samples was measured using the Aeroset (Abbott), the Hithachi 911 auto-analyzer, and a kit (total antioxidant status, Randox Lab. Ireland) containing the reduced molecule, ABTS [2-2' -Azinobis- (3-ethyl-benzothiazoline-6-sulphonic acid)].[17] The infarct volume was automatically calculated on the multidetector computed tomography (MCT) equipment; the results were expressed as cubic centimeters. The relationship between serum and CSF oxidative stress index and infarction volumes was studied.

Results were expressed as mean ± standard deviation. Correlation analysis was performed to determine the correlation between variables and Pearson's correlation coefficient was computed. SPSS 20.0 for Windows was used for statistical analysis. A p-value <0.05 was considered significant.

RESULTS

Of the 40 patients enrolled in the study with acute ischemic stroke, 18 (45%) were males and 22 (55%) were females. The youngest patient was 45 years old whereas the oldest was 74 years old; the mean age was 60.45±10.66. The overall mean CSF-OSI value was 1.80±0.47; the mean CSF-OSI value was 1.89±0.52 for male patients (n=18) and 1.73±0.42 for female patients (n=22). On the other hand, the overall mean serum-OSI value was 1.74±0.59; the mean serum-OSI value was 1.72±0.56 for male patients (n=18) and 1.75±0.62 for female patients (n=22).

The infarct volumes were measured in the control CBT, obtained after 48-72 hours. The overall mean infarct volume was 30.14±11.32 cm^3; the mean infarct volume was 30.70±11.3 cm^3 for the male patient group (n=18) and 29.60±11.5 cm^3 for the female patient group (n=22) (Table 1).

There was a significant positive correlation between the serum OSI values and the infarct volumes of all the patients who were enrolled in the study (n=40, r=0.513, p<0.001).

There was a significant positive correlation between the infarct volumes and the serum-OSI values of the male and female patients (respectively, n=18, p=0.033, r=0.504, n=22, p=0.014, r=0.569) (Table 2).

There was a significant positive correlation between the CSF-OSI values and the infarct volumes of all the patients who were enrolled in the study (n=40, r=509, p< 0,001). There was a significant positive correlation between the infarct volumes and the CSF-OSI values of the male and female patients (respectively, n=18, p=0.012, r=0.524, n=22, p=0.032, r=0.458) (Table 3).

TABLE 1: Serum-oxidative stress index, cerebrospinal fluid-oxidative stress index and cerebral infarct volume in males, females, and total patient groups.

Patient	Serum OSI	CSF OSI	CIV (cm^3)
Male (n=18)	1.72±0.56	1.89±0.52	30.70±11.3
Female (n=22)	1.75±0.62	1.73±0.42	29.60±11.5
Total (n=40)	1.74±0.59	1.80±0.47	30.14±11.3

Serum OSI: Serum oxidative stress index; CSF OSI: Cerebrospinal fluid oxidative stress index; CIV: Cerebral infarct volume.

TABLE 2: Correlation between serum oxidative stress index and cerebral infarct volume.

Patient	r	p
Male (n=18)	0.504	0.033
Female (n=22)	0.569	0.014
Total (n=40)	0.513	0.001

TABLE 3: Correlation between cerebrospinal fluid oxidative stress index and cerebral infarct volume.

Patient	r	p
Male (n=18)	0.524	0.012
Female (n=22)	0.458	0.032
Total (n=40)	0.509	0.001

The results of our study are relevant since they demonstrate increased oxidation in CSF and serum, as well as a positive correlation between increased oxidation and infarct volume in cases with acute ischemic stroke.

DISCUSSION

There have been important advances in stroke imaging, pathophsiology and treatment in recent years. Although CBT remains the initial imaging study in all patients that present with an acute neurologic deficit, it is used primarily to exclude the presence of hemorrhage and other stroke mimickers. MCT and MRI have been shown to be fast, reliable, and effective in improving stroke detection and to provide similar information.[18] Small vessel ischaemic disease may be evident as hypodense white matter lesions on CBT, but MRI is more sensitive to small vessel ischaemic changes, particularly if a FLAIR sequence is used.[19] Hence, ischemic subgroups with TIA and RIND were not involved in the study.

Investigating molecular and cellular toxic mechanisms leading to irreversible cell death caused by acute occlusion of blood flow is important to improve the present diagnostic and therapeutic approaches.[20] Free radicals are known to play an important role in the physiopathology of ischemic stroke. Free radicals have a very short half-life because of their considerable reactive nature. Thus, it is difficult to measure the free radicals directly. However, it is possible to indirectly determine their levels through antioxidant or oxidant levels.[21] Oxidative stress is based on either an increase in pro-oxidant production or the imbalance between antioxidant mechanisms. Human studies concerning cerebral oxidative stress and stroke are inadequate due to the methodological difficulties in measuring the free radical products. Reactive substances appear two days after the onset of cerebral ischemia.[22] Therefore, we evaluated our study subjects 48-72 hours after acute stroke. We assessed the oxidation capacity in the serum and concomitant CSF samples during this period of the oxidation process initiation.

In this study, we also used the total peroxides, which represent oxidants at the stage of the index formula. One of the parameters used in our study was the total antioxidant capacity. Collin A. R. *et al*. reported that antioxidant changes reflected unstable redox equilibrium during various pathological conditions.[23] In other words, antioxidants are in reaction with free radicals. Therefore, the concentrations of antioxidants or activity measurement have been used to determine the amount of oxidative stress.[24] The OSI, currently used as the ratio of oxidation products to the total antioxidants and as an indication of oxidative stress, was significantly increased both in the CSF and serum of our patient groups. In patients with ischemic stroke, oxidation is known to arise following neuronal damage and the antioxidant system intervenes in order to decompensate this oxidative injury.[15] The marked significance in the correlation between CSF and infarct volume, which was demonstrated in our study, may be associated with the relatively rapid development of the oxidative process in the CSF.

OSI represents a practical method in the follow-up of oxidative stress. Oxidative stress is known to appear during the first 48-72 hours of stroke. Advanced studies are required in order to investigate the state of oxidative stress and to investigate the period in which the antioxidant defense is active

and preventing oxidation. However, inability to directly measure oxidant and antioxidant molecules appears as a possible limitation in such studies. Hence, advanced procedures are required.

Additionally, these studies can also determine the efficiency of clinical neuroprotective agents. The index may become a marker to follow-up the damage of brain tissue.

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