The relationship of oxidative stress parameters with infarct volume and National Institutes of Health Stroke Scale in ischemic stroke

Abstract: Objective: What we know about the relationship between oxidative stress parameters and ischemic stroke is still limited and controversial. Our study aimed to investigate the relationships among ischemic lesion volume, National Institutes of Health Stroke Scale (NIHSS) values, and oxidant and antioxidant levels to determine whether oxidative stress parameters is effective on stroke severity in ischemic stroke patients.

Methods: The study included 34 patients with ischemic stroke and 34 volunteers with no active diseases. Total Oxidant Status (TOS), Total Antioxidant Status (TAS), thiol, paraoxonase, stimulated paraoxonase (stparaoxonase) and arylesterase were measured in blood samples collected on admission from patients diagnosed with ischemic stroke. The Oxidative Stress Index (OSI) was calculated. The same oxidative stress parameters were measured in the control group and compared with the patient group. Correlation between the oxidative stress parameters, the infarct volume and the NIHSS was studied. NIHSS was calculated when patients were admitted to the emergency department. The infarct volume was calculated using diffusion-weighted magnetic resonance imaging performed in the first 72–96 hours.

Results: TOS and OSI values were significantly higher in the case group than the control group. Paraoxonase, arylesterase, and thiol values were significantly lower in the case group than the control group. TAS and stparaoxonase values weren’t differed significantly between the case and control groups. There were significant negative correlations between the NIHSS value and both the paraoxonase value and stparaoxonase value. There were no significant correlations between the NIHSS value and the infarct volume and the TAS, TOS, OSI, arylesterase, and thiol values.

Conclusion: We concluded that change in oxidative stress balance in favor of oxidants could be a cause in the pathogenesis of ischemic stroke but oxidative stress alone can’t be sufficient in predicting the severity of stroke.

Keywords: Oxidative stress, ischemic stroke, infarct volume, NIHSS

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olmadığını tespit etmek için iskemik lezyon volümü, National Institutes of Health Stroke Scale (NIHSS), oksidan seviyeleri ve antioksidan seviyeleri arasındaki ilişkiyi araştırmayı amaçladık.

Metod: Çalışmaya 34 iskemik inmeli hasta ile aktif hastalıguna bulunmayan 34 gönlülü dahil edildi. Iskemik inme tanısı konulan hastalardan başvuru anında alınan kanlarda Total Oksidan Seviyesi (TOS), Total Antioksidan Seviyesi (TAS), tiyol, paraoxonaz, stimüle paraoxonaz ve arilesteraz çalışıldı. Oksidatif Stres İndeksi (OSI) hesaplandı. Kontrol grubundan alınan kanlarda da aynı oksidatif stres parametreleri çalışılarak hasta grubuya karşılaştırıldı. Oksidatif stres parametreleri ile NIHSS ve enfarkt volümü arasındaki korelasyon incelendi. Oksidatif stres parametreleri ile NIHSS ve enfarkt volümü arasındaki korelasyon incelendi. NIHSS hastaların acil servise başvuru anında hesaplandı. Enfarkt volümü ilk 72–96 saat içerisinde çekilen difizyon ağırlıklı MR’da hesaplandı.

Bulgular: Hasta grubunda TOS ve OSI değeri kontrol grubundan anlamlı olarak daha yüksekti. Hasta grubunda paraoxinaz, arilesteraz, tiyol değerleri kontrol grubundan anlamlı olarak daha düşüktü. Hasta ve kontrol grubunda TAS ve stparaoxinaz değeri anlamlı farklılık göstermedi. NIHSS ile paraoxinaz ve stparaoxonaz değerleri arasında anlamlı negatif korelasyon mevcuttu. NIHSS değeri ile TAS, TOS, OSI, arilesteraz, tiyol değerleri arasında anlamlı korelasyon yoktu. NIHSS değeri ile TAS, TOS, OSI, arilesteraz, tiyol, paraoxinaz ve stparaoxinaz değerleri arasında anlamlı korelasyon saptanmadı.

Sonuç: Oksidatif stres dengesinin oksidan lehine bozulması iskemik lezyonun patogenezinde etkili olabileceğini fakat oksidatif stresin tek başına inmenin şiddetini tahmin etmede yeterli olmayabileceğini düşünmektedir.

Anahtar Kelimeler: Oksidatif stres, iskemik inme, infarkt hacmi, NIHSS

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Introduction

While stroke is one of the major causes of permanent disability worldwide, it is the second most common cause of dementia, and third most common cause of mortality [1]. Further, it has become more important due to the gradual increase in the elderly population worldwide, which means high treatment costs and labor losses. Ischemic stroke is the most common form of all strokes [2].

Oxidative stress can be described as tissue damage resulting from an imbalance in antioxidant activity due to an increase in oxidant production for any reason, deficiency in the antioxidant defense mechanism, or a combination of both [3]. There is strong evidence that the production of free radicals during ischemia and reperfusion is important in the mechanisms that cause brain damage since brain tissue is especially prone to the deleterious effects of free radicals [4,5]. Experimental and clinical studies suggest that oxidative stress may play an important role in brain injury that is seen after a stroke [4,6].

It is known that both ischemic lesion volume and National Institutes of Health Stroke Scale scores (NIHSS) are important in predicting the post-stroke prognosis of patients. In this study, we aimed to investigate the effectiveness of both ischemic lesion volume and NIHSS score in predicting prognosis and oxidant and antioxidant levels, by calculating the correlations among these variables.

Materials and Methods

This study was conducted at the Emergency Department of Atatürk Teaching and Research Hospital between January 2012 and January 2013, after obtaining ethical committee approval. The study included 68 participants: 34 patients with ischemic stroke who were diagnosed with ischemic cerebrovascular disease according to World Health Organization criteria [7] and who agreed to participate in the study personally or through first degree relatives, and 34 volunteers with no active diseases. The patients’ age, sex, time from the start of symptoms until admission to the hospital, additional diseases, habits, complaints on admission, and physical examination findings were recorded. The control group was made up of individuals with no active diseases, and whose age and sex were consistent with the patient group.

Detailed neurological examinations were performed on all patients to determine their levels of consciousness, and the NIHSS score was calculated. The diagnosis was confirmed by cerebral tomography. Blood samples were collected immediately after diagnosis from patients with confirmed diagnoses of ischemic cerebrovascular disease in order to measure total oxidant status (TOS), total antioxidant status (TAS), thiol, paraoxonase, stimulated paraoxonase, and arylesterase. Obtained samples were centrifuged at 3000 g for 5 minutes and kept in a freezer at -80°C until the study.
Diffusion-weighted magnetic resonance imaging was performed in the first 72–96 hours in order to calculate the patients’ infarct volume.

Volume was calculated using apparent diffusion coefficient (ADC) maps, which we created from, diffusion-weighted image sequences with 5 mm thickness and 1 mm gap. Restricted diffusion areas were matched with infarct volume, and possible T2 shining effects were excluded by using ADC mapping.

**Exclusion criteria:** Patients who received a diagnosis of ischemic stroke more than 24 hours after the onset of symptoms, patients with no infarct detectable on computed tomography at the time of admission to the emergency department, and patients with lung disease (chronic obstructive and restrictive lung disease, lung cancer, collagen tissue disease, etc.), liver disease, and kidney disease were excluded from the study.

**Study limitations:** Penumbral effects could not be calculated on admission, since MRI could not be performed on the patients.

**Methods**

### Determination of serum TAS levels

TAS levels were measured using commercially available kits (Rel Assay Diagnostic®, RL0017). The novel automated method is based on the bleaching of the characteristic color of a more stable ABTS (2,2-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical cation by antioxidants. The assay has precision values, which are <3%. The results were expressed in mmol Trolox equivalent/L [8].

### Determination of serum TOS levels

TOS levels were measured using commercially available kits (Rel Assay Diagnostic®, RL0024). In the new method, the oxidants present in the sample oxidized the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction was enhanced by glycerol molecules abundantly present in the reaction medium. The ferric ion produced a colored complex with xylene orange in an acidic medium. The color intensity, which could be measured spectrophotometrically, was related to the total number of oxidant molecules present in the sample. The assay was calibrated with hydrogen peroxide, and the results were expressed in terms of micromolar hydrogen peroxide equivalent per liter (mmol H₂O₂ equivalent/L) [9].

### Calculation of Oxidative Stress Index (OSI)

OSI is calculated by dividing TOS/TAS, and a higher index indicates increased oxidative stress [10,11].

### Measurement of plasma levels of thiol

Plasma thiol levels were defined by measuring the color intensity of dark yellow-colored 5-thio-2-nitrobenzoic acid (TNB), which is produced during the oxidation of free thiol groups with Ellman’s reagent [5,50-ditioybis (2-nitrobenzoic acid), DTNB], at a wavelength of 412 nm [12].

### Measurement of paraoxonase, stimulated paraoxonase, and arylesterase activities

PON activity was determined using paraoxon as a substrate (Rel Assay Diagnostic®, RL0031) and measuring increases in the absorption at 412nm due to the formation of 4-nitrophenol, as already described [13]. The activity was measured at 25°C by adding 50 μL of serum to 1mL Tris-HCl buffer (100mM at pH 8.0) containing 2mM CaCl₂ and 5mM of paraoxon. The rate of generation of 4-nitrophenol was determined at 412 nm. Enzymatic activity was calculated by using a molar extinction coefficient of 17100M⁻¹ cm⁻¹. The stimulated PON activity was measured with addition of 1 mol/L NaCl to PON solution [14]. ARE activity was measured using phenylacetate as a substrate (Rel Assay Diagnostic®, RL0055). The serum was diluted 400 times in 100mM Tris-HCl buffer in a pH of 8.0. The reaction mixture contained 2.0mM phenylacetate (Sigma Chemical Co.) and 2.0mM CaCl₂ in 100mM Tris-HCl buffer in a pH of 8.0. Initial rates of hydrolysis were determined by following the increase in phenol concentration at 270nm at 37°C on a CE 7250 spectrophotometer (Cecil Instruments Limited, UK) [15]. Enzyme activities were expressed in international units (U) or kilounits (kU) per 1 liter of sera.

### Statistical method

Mean, standard deviation, rate and frequency values were used to describe the data. The distribution of variables was examined using the Kolmogorov-Smirnov test. The Mann-Whitney U test and Independent Sample t-test were used to analyze quantitative data. The Chi-square test was used to analyze qualitative data. Spearman’s correlation analysis was used to correlate variables. SPSS 21.0 was used for the analyses.
Results

There were no significant differences (p>0.05) between the ages and sexes of patients in the patient and control groups (Table 1).

44% (n=15) of the patients were admitted to the emergency department by ambulance, while 56% (n=19) were admitted as outpatients (Table 2). A review of patients’ complaints on admission revealed that 79% (n=27) were admitted with complaints of weakness, 26% (n=9) with speech disorder, 21% (n=7) with consciousness disturbance, 15% (n=5) with numbness, and 3% (n=1) with fatigue (Table 2).

TOS and OSI values were significantly higher (p<0.001) in the case group than in the control group. Paraoxonase, arylesterase, and thiol values were significantly lower (p<0.011, p<0.001, and p<0.012, respectively) in the case group than in the control group. The TAS and stparaoxonase values in the case and control groups showed no significant difference (p>0.05) (Table 3).

There were significant positive correlations (p<0.05) between NIHSS value and both infarct volume and admission time. There were significant negative correlations between NIHSS value and both paraoxonase and stparaoxonase values in the case and control groups showed no significant difference (p>0.05) (Table 3).

Table 1: Demographic data of the patient group and the control group.

|                               | Patient group Mean±SD | Control group Mean±SD | p   |
|-------------------------------|-----------------------|-----------------------|-----|
| Age                           | 69.91±11.33           | 71.41±5.53            | 0.490|
| Sex                           |                       |                       |     |
| Female                        | 17 50                 | 20 59                 | 0.465|
| Male                          | 17 50                 | 14 41                 |     |

Table 2: Clinical and background details of the patient group.

| Type of arrival | Add disease | n   | %   | n   | %   |
|-----------------|-------------|-----|-----|-----|-----|
| By ambulance    | DM          | 15  | 44  | 10  | 29  |
| Outpatients     | HT          | 19  | 56  | 23  | 68  |
| On admission    | CVD         | 7   | 21  | 8   | 24  |
| Loss of consciousness | CHD | 7  | 21 | 10 | 29 |
| Loss of strength | Arrhythmia  | 27  | 79  | 5   | 15  |
| Weakness        | Other       | 1   | 3   | 11  | 32  |
| Numbness        |             | 5   | 15  |     |     |
| Speech impairment |            | 9   | 26  |     |     |
| Smoking         |             | 4   | 12  |     |     |
| Alcohol use     |             | 1   | 3   |     |     |

Table 3: Comparisons of oxidative stress parameters between the patient group and control group.

|                               | Patient group Mean±SD | Control group Mean±SD | p   |
|-------------------------------|-----------------------|-----------------------|-----|
| TAS (mmol Trolox eq/L)        | 2.36±1.00             | 2.31±0.31             | 0.218|
| TOS (μmol H2O2 Eq/L)          | 3.73±2.49             | 1.32±1.23             | <0.001|
| OSI (Arbitrary Unit)          | 1.67±1.18             | 0.58±0.54             | <0.001|
| Paraoxonase (U/L)             | 130.10±79.68          | 179.20±81.30          | 0.011|
| St Paraoxonase (U/L)          | 337.17±219.19         | 439.07±247.50         | 0.077|
| Arylesterase (U/L)            | 134.46±47.77          | 187.52±48.63          | <0.001|
| Thiol (μmol/L)                | 133.11±43.67          | 158.24±36.10          | 0.012|

Independent samples t test/Mann-whitney U test.
nase values (p<0.031 and p<0.029, respectively). There were no significant correlations (p>0.05) between NIHSS value and TAS, TOS, OSI, arylesterase, and thiol values (Table 4).

There were no significant correlations (p>0.05) between infarct volume value and the following variables: admission time, TAS, TOS, OSI, arylesterase, thiol, paraoxonase, and stparaoxonase (Table 4).

There were no significant correlations (p>0.05) between admission time and TAS, TOS, OSI, arylesterase, thiol, paraoxonase, and stparaoxonase values (Table 4).

The NIHSS, infarct volume, admission time, TAS, TOS, OSI, arylesterase, thiol, paraoxonase and stparaoxonase values of patients admitted by 112 emergency ambulance service did not differ significantly from those admitted as outpatients (p>0.05).

Discussion

Lesion volume is believed to be an important parameter reflecting the primary pathological condition, and the extent of this pathological condition relates to neurological deficits and functional outcome [16]. Hence, infarct volume may serve as a predictor of the severity of neurological impairments such as paresis (i.e., neurological deficits) and functional outcomes such as activities of daily living dependency after a stroke [17]. The NIHSS is an 11-category (15-item) neurologic evaluation (score range of 0 to 42) that is quick to use (5 to 10 minutes), yields reproducible results, has a high inter-rater reliability, and provides a score that correlates with infarct volume [18]. In this study, our aim was to compare NIHSS values and infarct volume effectiveness in predicting prognosis with TAS, TOS, paraoxonase, stimulated paraoxonase, arylesterase and thiol levels, and to investigate the effects of these markers in prognosis. There was a positive correlation between NIHSS values and infarct volume in our study. While there was a significant negative correlation between the NIHSS value and paraoxonase and stparaoxonase values, there were no significant correlations between NIHSS values and TAS, TOS, OSI, arylesterase, or thiol values. In addition, no significant correlations were found between infarct volume and TAS, TOS, OSI, arylesterase, thiol, paraoxonase and stparaoxonase values.

Although it is possible to separately measure the antioxidants in plasma, due to the high number of antioxidants and their in vivo interactions, it is accepted that tests showing TAS are more valuable in estimating oxidative stress [19]. This is why measuring total antioxidant capacity, which yields a total antioxidant value, has become the commonly-used method in predicting the antioxidant capacity of blood, rather than measuring antioxidant markers one by one [8,20]. Further, in determining whether the oxidant/antioxidant balance in the blood is in favor of oxidants or antioxidants, OSI alone is considered to be much more sensitive than the use of oxidants and/or antioxidants. In our study, TAS and OSI were found to be significantly higher in the patient group than the control group, but there was no significant correlation between infarct volume and NIHSS values. In light of these results, we conclude that oxidative stress can play a role in the pathogenesis of ischemic stroke but is ineffective in predicting patients’ long-term prognoses.

Both experimental and clinical studies show that oxidative stress plays an important role in brain damage after stroke [4,21]. However, although the effects of free radicals are very well known, the role of antioxidant mechanisms in the process is still unclear [22]. Different from the TAS measurement in our study, Leinonen et al. reported a significant correlation between the total peroxyl radical-trapping potential of plasma, which they calculated and

Table 4: Correlations among NIHSS values, infarct volume, admission time and oxidative stress parameters.

|                      | NIHSS     | Infarct volume | Admission time (hour) | TAS     | TOS     | OSI     | PON     | St PON  | ARE     | Thiol   |
|----------------------|-----------|----------------|----------------------|---------|---------|---------|---------|---------|---------|---------|
| r                    | 0.456     | 0.560          | -0.168               | -0.220  | -0.172  | -0.371  | -0.375  | -0.145  | -0.145  | -0.055  |
| p                    | 0.007     | 0.001          | 0.342                | 0.211   | 0.332   | **0.031** | **0.029** | 0.414   | 0.758   |         |
| r                    | -         | 0.227          | 0.027                | 0.060   | 0.055   | -0.192  | -0.191  | -0.056  | -0.087  |         |
| p                    | -         | 0.197          | 0.880                | 0.737   | 0.756   | 0.277   | 0.278   | 0.751   | 0.626   |         |
| r                    | -         | -              | -0.191               | -0.070  | -0.007  | -0.332  | -0.305  | 0.110   | 0.017   |         |
| p                    | -         | -              | 0.279                | 0.693   | 0.969   | 0.055   | 0.079   | 0.536   | 0.923   |         |

Spearman/Pearson correlation. TAS: mmol Trolox eq/L; TOS: μmol H₂O₂ Eq/L; PON: U/L; St PON: U/L; Thiol: μmol/L; ARE: U/L.
considered as TAS, and both infarct volume and NIHSS [23]. Other studies have reported that TAS decreased in ischemic stroke [24–26]. In our study, despite the fact that antioxidants such as paraoxonase, arylesterase and thiol decreased one by one between the patient group and the control group, there were no significant differences in their TAS values. There were also no significant correlations between TAS and NIHSS and infarct volume.

The thiol groups have the highest plasma concentration due to the high level of plasma protein in adults [27]. The study by Leinonen et al. described above also found a significant correlation between thiol levels and infarct volume and NIHSS [23]. They concluded that for other antioxidants such as α-tocopherol, uric acid, and ascorbic acid, as well as thiol, the increase in plasma activity could protect the brain from the neurological damage caused by stroke [23]. In our study, thiol levels were significantly lower in the patient group than the control group. However, there was no significant correlation between thiol levels and infarct volume and NIHSS.

The paraoxonase enzyme is a calcium-dependent aromatic hydroxylase. It is believed to play an important role in protecting LDL and HDL from oxidation, as an antioxidant effect against lipid peroxidation in cellular membranes [28]. It has been reported that paraoxonase activity decreased in patients with ischemic stroke; in fact, it could be effective both in physiopathology and as a risk factor in acute stroke [26,29,30]. The paraoxonase levels of our patient group were also significantly lower than those of our control group. Further, there was no correlation between paraoxonase and infarct volume, with a significant negative correlation between paraoxonase and NIHSS. These data suggest that paraoxonase is important in physiopathology but that its relationship to prognosis is not clear.

Wide variations in paraoxonase-1 activities (up to 13-fold) were described even among individuals with the same genotype 112. At present, arylesterase activity is considered to be one of the paraoxonase-1 functions and the most reliable indicator of antioxidant/anti-inflammatory efficacy [31]. In studies where arylesterase was measured in ischemic stroke, it has been shown that arylesterase levels decreased in patient groups [32]. In our study, too, arylesterase levels in the patient group were significantly lower than in the control group, consistent with the literature.

Conclusion

As reported in many previous studies, We concluded that change in oxidative stress balance in favor of oxidants could be a cause in the pathogenesis of ischemic stroke, but that particularly due to the absence of significant correlations between TAS, TOS and OSI and infarct volume and NIHSS values, oxidative stress alone can't be sufficient in predicting the severity of stroke.

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