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The relation between oxidative stress parameters, ischemic stroke, and hemorrhagic stroke

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Background/aim: The aims of this study were to investigate the significance of oxidative stress parameters in the pathogenesis of ischemic stroke and hemorrhagic stroke and to investigate their effects on stroke severity using the National Institutes of Health Stroke Scale (NIHSS).

Materials and methods: A total of 92 patients, including 74 with ischemic stroke and 18 with hemorrhagic stroke, and 75 volunteers were enrolled in the study. Total oxidant status (TOS), total antioxidant status (TAS), paraoxonase, stimulating paraoxonase, arylesterase, and thiol levels were measured in both the patient and volunteer groups. NIHSS and oxidative stress index (OSI) scores were calculated.

Results: TOS and OSI levels were significantly higher in the ischemia and hemorrhagic stroke groups than in the control group ($P < 0.05$). Arylesterase and thiol levels were significantly lower in the ischemia group than the control group ($P < 0.05$). No significant correlation was found between NIHSS score and TAS, TOS, OSI, paraoxonase, arylesterase, stimulated paraoxonase, and thiol levels ($P > 0.05$).

Conclusion: Oxidative stress may play a role in the pathogenesis of both ischemic stroke and hemorrhagic stroke in terms of oxidants. We do not think that oxidative stress has any effect in determining stroke severity in either type of stroke.

Key words: Oxidative stress, ischemic stroke, hemorrhagic stroke, National Institutes of Health Stroke Scale

1. Introduction

Stroke is a medical emergency situation that may cause loss of brain function and even death. This condition is the second most common cause of death and the most common cause of impairment among adults in the West (1). Strokes are classified into two groups according to their pathology: ischemic stroke and hemorrhagic stroke. Ischemic strokes make up 87% and hemorrhagic strokes 13% of all strokes (2).

Electron acceptor molecules in biological systems are known as free oxygen radicals (3). Active oxygen derivatives of free radicals are referred to as oxidants. Oxidants change the structure and function of target molecules by receiving electrons from them. Oxidants also affect cell membranes and genetic material such as DNA, RNA, and various enzymatic events, and they lead to cell damage during ischemia and reperfusion. Various antioxidants protect the organism from the harmful effects of oxidants by destroying them (4). In the organism, imbalance between the oxidant and antioxidant systems caused by production of excessive amounts of free oxygen radicals or insufficient antioxidants is called oxidative stress (4). Oxidative stress has been shown to play a role in the occurrence and progression of many local and systemic diseases.

Experimental and clinical studies suggest that oxidative stress plays an important role in brain injury that follows a stroke (5,6). However, although the effect of free oxygen radicals in the process is well-known, the role of antioxidant mechanisms has not been clarified (7). There are very few studies investigating the effects of oxidative stress in hemorrhagic stroke (8). The aims of this study were to investigate the significance of oxidative stress parameters in the pathogenesis of ischemic and hemorrhagic stroke and to investigate their effects on stroke severity using the National Institutes of Health Stroke Scale (NIHSS).

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2. Materials and methods

2.1. Patients

This study was conducted in the Ankara Atatürk Training and Research Hospital emergency service between January and November 2013, after receiving approval from the ethics committee and informed consent from the patients or their first-degree relatives.

The study group included a total of 92 patients, who were diagnosed with stroke according to World Health Organization criteria. Seventy-four patients had ischemic stroke and 18 had hemorrhagic stroke (nontraumatic subarachnoid hemorrhage and intraparenchymal hemorrhage). The control group included 75 healthy volunteers who were not suspected of having stroke or transient ischemic attack and did not have any of the exclusion criteria.

All patients underwent detailed neurological examinations to determine their level of awareness; cerebral tomography was completed. Immediately after the diagnoses were confirmed by cerebral tomography and neurological examination, the NIHSS score was calculated, and blood samples were taken for examining total oxidant status (TOS), total antioxidant status (TAS), paraoxonase, stimulated paraoxonase, arylesterase, and thiol. Blood samples were taken from the control group in order to study the same oxidative stress parameters. The samples were centrifuged at 3000 rpm for 5 min and stored at –80 °C in a deep freezer. All samples were dissolved and analyzed simultaneously during the study.

Patient cards included demographical data (age, sex), duration between onset of symptoms and hospital admission, symptoms and physical examination findings on admission, additional diseases, and habits (alcohol, tobacco). NIHSS scores were calculated according to the modification of Meyer et al. (9).

2.2. Exclusion criteria

Patients were excluded from the study on the basis of the following criteria: delay of more than 24 h between onset of symptoms and diagnosis of stroke; no bleeding area or infarct identified in the tomography taken on admission to the emergency room; bleeding or infarcts due to reasons such as trauma, tumor, or infection; lacunar infarct; and pulmonary (chronic obstructive and restrictive lung disease, lung cancer, collagen vascular disease, etc.), liver, or kidney diseases.

2.3. Limitations of the study

Although its proportion was representative of the number of hemorrhagic stroke patients in the population, the hemorrhagic stroke group in our study was small. Moreover,

infarct volume, which has a prognostic value in ischemic stroke, was not calculated.

2.4. Methods

2.4.1. Determination of serum TAS levels

TAS levels were measured using commercially available kits (Rel Assay, Turkey). 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 excellent precision values that are lower than 3%. The results were expressed as mmol Trolox equivalent/L (10).

2.4.2. Determination of serum TOS levels

TOS levels were measured using commercially available kits (Rel Assay, Turkey). With 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 xylenol orange in an acidic medium. Color intensity, which could be measured spectrophotometrically, was related to the total amount 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 (mol H_2O_2 equivalent/L) (11).

2.4.3. Calculation of OSI

OSI is calculated by dividing TOS by TAS, and a higher index indicates increased oxidative stress (12,13).

2.4.4. 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 reagent [5,5'-dithiobis (2-nitrobenzoic acid), DTNB] at a wavelength of 412 nm (14).

2.4.5. Measurement of paraoxonase, stimulated paraoxonase, and arylesterase activities

Paraoxonase activity was determined using paraoxon as a substrate and was measured by increases in absorbance at 412 nm due to the formation of 4-nitrophenol, as described previously (15). The activity was measured at 25 °C by adding 50 µL of serum to 1 mL of Tris-HCl buffer (100 mM at PH 8.0) containing 2 mM $CaCl_2$ and 5 mM paraoxon. The rate of generation of 4-nitrophenol was determined at 412 nm. Enzymatic activity was calculated by using molar extinction coefficient 17,100 M^{-1} cm^{-1}. Arylesterase activity was measured using phenyl acetate as a substrate. The serum was diluted 400 times in 100 mM Tris-HCl buffer, pH 8.0. The reaction mixture contained 2.0 mM phenyl acetate (Sigma Chemical Co.) and 2.0 mM $CaCl_2$ in 100 mM Tris-HCl buffer, pH 8.0. Initial rates of hydrolysis were determined by following the increase of phenol concentration at 270 nm at 37 °C on a CE 7250 spectrophotometer (Cecil Instruments, UK) (16). Enzyme

activities were expressed in international units (U) or kilounits (kU) per 1 L of sera.

2.5. Statistical analysis

Data from descriptive statistics were analyzed using mean, standard deviation, rate, and frequency values. The distribution of variables was checked for normality with the Kolmogorov–Smirnov test. The Mann–Whitney U test and independent samples t-test were used in the analysis of quantitative data. The chi-square test was used in the analysis of qualitative data. Spearman's rho correlation was used for correlation analyses. SPSS 21.0 was used for statistical analysis.

3. Results

Age and sex did not differ between the patient groups and the control group (P > 0.05) (Table 1).

The number of patients brought by ambulances was 47 (51%) and the number of patients arriving at the emergency department by their own transportation was 45 (49%) (Table 2). The most common complaints on admission were weakness (n = 66, 66%), loss of consciousness (n = 25, 27%), speech difficulty (n = 20, 22%), numbness (n = 11, 12%), and fatigue (n = 1, 1%), respectively (Table 2). According to patients' history, 18 patients (20%) were smokers and 2 patients (2%) consumed alcohol. Regarding patients' medical history, 59 patients (64%) had hypertension (HT), 29 patients (32%) had atherosclerotic heart disease, 24 patients (26%) had diabetes mellitus, 17 patients (18%) had previous cerebrovascular disease (CVD), 13 patients (14%) had arrhythmia, and 26 patients (28%) had other comorbidities (Table 2).

TOS and OSI values were significantly higher in the patient group than in the control group (P < 0.05). Arylesterase and thiol values were significantly lower in the patient group than the control group (P < 0.05). TAS, stimulated paraoxonase, and paraoxonase levels did not differ significantly among the groups (P > 0.05) (Table 3).

There was a negative correlation between NIHSS score and admission period (P < 0.05). There were no significant correlations between NIHSS score and TAS, TOS, OSI, paraoxonase, arylesterase, stimulated paraoxonase, and thiol values (P > 0.05) (Table 4).

There were no significant correlations between admission period and TAS, TOS, OSI, paraoxonase, arylesterase, stimulated paraoxonase, and thiol values (P > 0.05) (Table 4).

Table 1. Demographic data of the patient and control groups.

		Patient group	Control group	P
		Mean ± SD / n %	Mean ±SD / n %	
Age		71.80 ± 12.33	72.36 ± 6.10	0.705
Sex	Female	47 51	38 51	0.957
	Male	45 49	37 49	

Independent samples t-test/chi-square test.

Table 2. Characteristics of the patient group.

		n	%			n	%
Type of arrival	With ambulance	47	51	Smoking		18	20
	Outpatients	45	49	Alcohol use		2	2
On admission	Loss of consciousness	25	27	Add. disease	DM	24	26
	Loss of strength	66	72		HT	59	64
	Weakness	1	1		CVD	17	18
	Numbness	11	12		CHD	29	32
	Speech impairment	20	22		Arrhythmia	13	14
Admission time (mean ± SD)		8.03 ± 5.95			Other	26	28
Type	Ischemia	74	80	NIHSS		6.61 ± 6.90	
	Hemorrhage	18	20				

Table 3. Patient groups and control group were compared in terms of oxidative stress parameters.

	Patient group	Control group	P
	Mean ± SD	Mean ± SD	
TAS	2.32 ± 0.65	2.34 ± 0.30	0.326
TOS	2.86 ± 1.86	2.10 ± 1.58	0.005
OSI	1.26 ± 0.82	0.91 ± 0.70	0.004
Paraoxonase	132.23 ± 78.95	150.77 ± 89.00	0.276
St. paraoxonase	343.64 ± 217.13	389.35 ± 250.69	0.401
Arylesterase	138.17 ± 58.89	180.96 ± 62.90	0.001
Thiol	122.77 ± 47.38	151.03 ± 40.78	0.001

Independent samples t-test / Mann–Whitney U test.

Table 4. Admission period and oxidative stress parameters compared to NIHSS.

		Admission time (hours)	TAS	TOS	OSI	PON	St. PON	ARE	Thiol
Admission time (hours)	r	-	0.022	–0.086	–0.107	0.128	0.134	–0.004	0.048
	P		0.832	0.413	0.311	0.223	0.202	0.970	0.648
NIHSS	r	–0.257	–0.075	0.109	0.130	–0.143	–0.132	–0.102	–0.164
	P	0.003	0.476	0.303	0.218	0.173	0.211	0.334	0.118

Spearman–Pearson correlation.

There were no significant differences between the subgroups of patients with ischemia and bleeding in NIHSS, TAS, TOS, OSI, arylesterase, thiol, paraoxonase, and stimulated paraoxonase values ($P > 0.05$) (Table 5).

There were no significant differences between the patients with ischemic stroke and the control group in terms of TAS, paraoxonase, and stimulated paraoxonase values ($P > 0.05$). TOS and OSI values were significantly higher in the ischemia group than in the control group ($P < 0.05$). Arylesterase and thiol values were significantly lower in the ischemia group than in the control group ($P < 0.05$) (Table 5).

TAS, paraoxonase, stimulated paraoxonase, arylesterase, and thiol values did not differ significantly between the hemorrhagic stroke patients and the control group ($P > 0.05$). TOS and OSI values were significantly higher in the hemorrhagic stroke patients than in the control group ($P < 0.05$) (Table 5).

4. Discussion

Since it has been shown that oxidative stress parameters were effective in the occurrence, progression, and complications of many local and systemic diseases, a number of studies on oxidative stress parameters have been conducted. Although oxidant and antioxidant levels in stroke patients have been widely studied, their role, especially in pathogenesis, is still controversial. In addition, there are significantly fewer studies on hemorrhagic stroke patients than on ischemic stroke patients; studies dealing with and comparing both of these groups are extremely limited (7,8). In our study, we investigated oxidative stress parameters in both ischemic stroke and hemorrhagic stroke patients. According to the results of our study, TOS and OSI levels were increased in both ischemic and hemorrhagic stroke. In hemorrhagic stroke, the total antioxidant level and other antioxidant levels did not change significantly, whereas thiol and arylesterase levels were higher in ischemic stroke. No change was detected in total antioxidant and other antioxidant levels.

Parizadeh et al. showed that the prooxidant/antioxidant balance increased in the serum of patients with stroke; however, there was no difference between the ischemic and hemorrhagic stroke subgroups. Additionally, they showed that oxidative parameters were not useful in predicting the 6-month prognosis (7). In another study it was shown that TOS levels increased in the serum of patients with

Table 5. Comparison of hemorrhagic stroke, ischemic stroke, and control groups in terms oxidative stress parameters.

	Ischemia	Hemorrhage	Control	Isc-Hem	Isc-Cont	Hem-Cont
	Mean ± SD	Mean ± SD	Mean ± SD	P	P	P
NIHSS	8.15 ± 5.93	7.56 ± 6.16	- -	0.671	-	-
Admission time	6.81 ± 6.83	5.78 ± 7.34	- -	0.256	-	-
TAS	2.35 ± 0.71	2.21 ± 0.23	2.34 ± 0.30	0.598	0.492	0.104
TOS	2.81 ± 1.93	3.07 ± 1.55	2.10 ± 1.58	0.558	0.015	0.021
OSI	1.25 ± 0.85	1.31 ± 0.70	0.91 ± 0.70	0.779	0.009	0.032
Paraoxonase	134.3 ± 78.90	123.76 ± 80.89	150.77 ± 89.0	0.448	0.412	0.243
Stimulated paraoxonase	347.5 ± 215.8	327.37 ± 227.8	389.35 ± 250.6	0.506	0.506	0.403
Arylesterase	132.2 ± 48.86	162.52 ± 86.80	180.96 ± 62.90	0.169	0.001	0.304
Thiol	122.6 ± 41.33	123.30 ± 68.41	151.03 ± 40.78	0.969	0.001	0.114

Independent samples t-test / Mann–Whitney U-test.

hemorrhagic stroke in the acute phase. Nevertheless, there was no significant correlation between TOS, total hematoma volume, and Glasgow Coma Scale (GCS) score (8). Kotan et al. showed that OSI and infarct volume were correlated, and they suggested that oxidative stress plays a role in the pathogenesis of ischemic stroke (17). Aygul et al. also suggested that oxidative stress was involved in the pathogenesis of ischemic stroke (18). In our study, TOS and OSI levels were significantly higher in all stroke patients, both with ischemic stroke and hemorrhagic stroke, than in the control group. There was no difference between the ischemic stroke and hemorrhagic stroke subgroups. There was no significant difference between NIHSS score, which is an important indicator of stroke severity, and TOS and OSI in either stroke groups. In light of the present findings, we think that oxidants formed before or during ischemia might damage neurons in both ischemic stroke and hemorrhagic stroke and may play a role in their pathophysiology. Nevertheless, oxidant levels are not suitable for determining stroke severity.

TAS level indicates the level of total antioxidants in the body; therefore, it better reflects whether oxidative stress is caused by excess antioxidants. This measure is more suitable than measuring individual substances with antioxidant properties (19). Çevik et al. found that malondialdehyde and TAS increased in the plasma of patients with hemorrhagic stroke in the acute phase, but these measures were not correlated with GCS. They suggested that although antioxidants play a role in the pathogenesis of hemorrhagic stroke, oxidative stress markers do not have any effect on the severity of stroke (8). Similarly, Aygul et al. did not find any correlation between oxidative stress parameters and GCS in their study (20). In another study examining antioxidant ascorbic acid, a positive correlation with GCS score and a negative correlation with the volume of the hematoma were reported (21). Once more, Leinonen et al. reported a significant relationship between total plasma peroxyl radical-trapping potential, which they accepted to be TAS. In addition, they measured and calculated TAS and NIHSS in a different way than in this study (22). Lagowska-Lenard et al. found a reduction in TAS in stroke patients when compared to the control group (23). Other studies also reported a decrease in TAS levels in ischemic stroke, whereas Sheikh et al. reported no change in TAS levels in patients with ischemic stroke (24–26). In our study, TAS levels did not change significantly, neither in the hemorrhagic nor in the ischemic stroke group, and there was no correlation between TAS and NIHSS scores. On the basis of these results, we suggest that oxidative stress that is in equilibrium in the acute phases of both ischemic and hemorrhagic strokes becomes unbalanced due to an increased oxidant level rather than a decreased antioxidant level; therefore, antioxidants do not have a role in the pathogenesis of stroke. In addition, the absence of a statistically significant relationship between NIHSS score and TAS suggests that antioxidant levels are not effective in determining stroke severity.

Paraoxonase is an enzyme that exists in the structure of HDL cholesterol and inhibits lipoprotein oxidation by hydrolyzing lipid peroxides in oxidized LDL. The paraoxonase enzyme, which exists within circulating HDL, inhibits LDL oxidation by inhibiting the release of superoxide anion from macrophages and reduces the formation of oxidized LDL. Thus, inflammatory processes that are induced by oxidized LDL in endothelial cells are

avoided (27). Several studies investigating the relationship between stroke and paraoxonase levels in ischemic stroke reported varying results, depending on the society in which the study was conducted. However, in almost all of these studies no correlation between paraoxonase levels and hemorrhagic stroke was found (25,28–30). In addition, Acer et al. did not find any correlation between paraoxonase and NIHSS in their study of ischemic stroke, although they discovered a negative correlation between TAS and NIHSS (25). In our study, paraoxonase levels showed no significant changes in the hemorrhagic or ischemic stroke groups. We suggest that the varying results in our study and other studies depend on polymorphic differences of paraoxonase enzyme activity with respect to individuals.

Paraoxonase activity may be stimulated by NaCl, and as a result stimulated paraoxonase is produced (31). Although the significance of paraoxonase activity stimulated by NaCl is not clearly understood, it is used in detecting the paraoxonase-1 phenotype (32). Two types of paraoxonase phenotype, A and B, have been identified in the studies conducted after it was understood that the paraoxonase-1 and arylesterase activities of the paraoxonase enzyme change under different pH and salt concentrations. Phenotype B, which is responsive to NaCl stimulation, has a high enzyme activity against paraoxonase, whereas arylesterase activity decreases with NaCl stimulation (32). In our study, stimulated paraoxonase levels, in parallel with paraoxonase levels, did not change significantly in either group.

Arylesterase is an antioxidant enzyme coded by the same gene as paraoxonase; however, unlike paraoxonase, it does not show genetic polymorphism. Measurements of oxidative stress in many systemic diseases have revealed a very significant correlation between serum levels of arylesterase and these diseases (33). In our study, arylesterase levels were significantly lower in the ischemic stroke group, whereas no significant change in hemorrhagic stroke was observed.

The term 'thiol' represents compounds containing sulfur (-SH) groups. Plasma thiols have prooxidant or antioxidant effects on physiological events, but they are generally considered to be antioxidants (34,35). Plasma thiol includes cysteine as the largest compound, followed by homocysteine and glutathione, respectively. It is accepted that the -SH group in thiols is protective against oxidative stress. Prooxidant effects induced by thiol compounds have been reported in renal ischemia, liver failure, and diseases of cardiovascular and cerebrovascular tissues (36,37). In their study, Leinonen et al. found a significant correlation between thiol levels and NIHSS. However, Musumeci et al. did not find any correlation between NIHSS and the SH groups (22,38).

In our study, thiol levels in ischemic stroke patients were significantly lower than in the control group, whereas they did not change in patients with hemorrhagic stroke. According to these results, thiol levels may be involved in the pathogenesis of ischemic stroke but not in hemorrhagic stroke. Due to the lack of any correlation between oxidative stress parameters and NIHSS for either type of stroke, we suggest that it is not useful in predicting prognosis.

Based on the finding of higher TOS and OSI values, we suggest that oxidative stress is in the direction of oxidation in the pathogenesis of both ischemic stroke and hemorrhagic stroke. Because the TAS and other antioxidant levels we studied showed no significant changes, we suggest that antioxidants do not play a role in the pathogenesis of hemorrhagic stroke. In ischemic stroke, TAS, paraoxonase, and stimulated paraoxonase levels showed no significant change, although thiol and arylesterase levels showed a significant decrease. Therefore, we suggest that they play a partial role in pathogenesis. Finally, due to the lack of any correlation between oxidative stress parameters and NIHSS for either type of stroke, we suggest that oxidative stress parameters have no role in determining stroke severity.

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