

## Evaluation of serum oxidant/antioxidant balance in patients with acute stroke

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### Abstract

**Objectives:** To investigate the alterations in the oxidant-antioxidant balance in patients with acute ischaemic stroke, and to locate any correlation between oxidant/antioxidant parameters and the National Institute of Health Stroke Scale.

**Methods:** The case-control study was conducted at the Neurology Department of Dicle University Medical Faculty, Diyarbakir, Turkey, from June 2010 to June 2011. Blood samples were obtained from 53 patients with ischaemic stroke and 40 healthy controls without any history of ischaemic stroke or systemic disease. Venous blood was obtained within 24 hour after stroke onset. Serum malondialdehyde, total anti-oxidant capacity, paraoxanase and superoxide dismutase were measured. SPSS 11.5 used for statistical analysis.

**Results:** There was no difference between the cases and the controls regarding age [64.5±15.8 and 66.3±13.9 respectively], gender [27 (51%) / 26 (49%), and 19 (48%) / 21 (52%) respectively], obesity [15 (28.3%) and 13 (37.5%), respectively], and hypertension [30 (56.6%) and 23 (57.5%), respectively]. The cases had higher concentrations of malondialdehyde (147.3±59.3 vs. 112.4±28.5 nmol/gr protein,  $p<0.001$ ), and superoxide dismutase (4.40±0.79 vs. 3.35±0.51,  $p<0.001$ ) compared to the controls. However, the cases had lower concentrations of paraoxanase (23.2±23.7 vs 64.7±52.6,  $p<0.001$ ), total anti-oxidant capacity (0.77±0.38 vs. 0.95±0.30,  $p<0.015$ ), and nitric oxide (10.8±7.1 vs. 17.5±2.4  $\mu\text{mol/gr}$  protein,  $p<0.001$ ), compared to the controls. In the stroke group, a significant negative correlation was found between the National Institute of Health Stroke Scale and total anti-oxidant capacity activity ( $p<0.021$ ,  $r = -0.32$ ).

**Conclusion:** The results support the hypothesis that sufficient anti-oxidant capacity has a beneficial effect on the clinical severity of acute ischaemic stroke.

**Keywords:** Acute ischaemic stroke, Pathogenesis, Total antioxidant status, Malondialdehyde. (JPMA 63: 590; 2013)

### Introduction

Stroke is a medical emergency situation that may lead to 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 Western world.<sup>1</sup> Ischaemic stroke is a heterogeneous syndrome occurring due to multiple disease mechanisms caused by the tissue damage resulting from a disturbance in the cerebral blood circulation. The degree of the focal cerebral injury is influenced by various molecular mechanisms.<sup>2</sup> Brain ischaemia is described as a condition where the fractional oxygenation of the brain tissue is inadequate to maintain the cellular oxidative metabolism, leading to metabolic changes and possible cell deaths.<sup>3</sup> In addition, the ischaemia and reperfusion that follow the stroke has been demonstrated to be associated with certain reactions mediated by the free radicals that may potentially cause neurone death.<sup>4</sup> Oxidative stress is one of the mechanisms contributing to the neuronal damage potentially induced through the ischaemia and reperfusion, and the antioxidant activity in the blood may be an important

factor providing protection against the neurological damage caused by the oxidative stress related to the stroke.<sup>5</sup> Although the balance is in favour of the anti-oxidant activity against free radical generation in healthy individuals, but in case of ischaemic stroke, the balance between the reactive oxygen species and anti-oxidant activity shifts towards the free radicals that cause oxidative stress.<sup>6</sup> Therefore, improved total anti-oxidant capacity (TAC) following an acute stroke may provide protection against the negative effects of free radical production during ischaemia and reperfusion.<sup>7</sup> Malondialdehyde (MDA), which has large-scale usage as an oxidative stress biomarker, has come into the spotlight due to its ability to react with the lipoproteins.<sup>8</sup> A correlation between the MDA levels and the infarct, the severity of the clinical stroke, and the patient outcome has been observed.<sup>9,10</sup> An increase in MDA levels may be more important in the brain compared to other tissues due to the brain's defencelessness against the reactive oxygen species, resulting from its high oxygen requirement, high lipid content and relatively low anti-oxidant defence mechanisms.<sup>11</sup>

Paraoxonase (PON1) is an ester hydrolase with arylesterase and paraoxonase activities. PON1 has been recently demonstrated to participate in various activities besides the lipid and lipoprotein metabolism based on its anti-

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atherogenic and anti-oxidant features.<sup>12</sup> The enzyme superoxide dismutase (SOD) catalyses the reaction where the superoxide anion is transformed into H<sub>2</sub>O<sub>2</sub> and molecular oxygen. The main purpose of this enzyme is to protect the cellular structures from the harmful effects of the superoxide.<sup>13</sup> The TAC reflects the total activity of the total substances of anti-oxidant nature in the serum.<sup>14</sup> There are only a limited number of studies that have investigated both the oxidant parameters like MDA and anti-oxidant parameters such as TAC in patients with ischaemic stroke together. The aim of this study was to investigate the interaction between the serum anti-oxidants (TAC, SOD, PON1) and MDA, and possible correlation between oxidant/antioxidant parameters and the clinical severity of the stroke.

### Patients and Methods

The case-control study comprised 53 acute cerebral ischaemic infarct patients admitted to the neurology department of Dicle University Medical Faculty, and 40 healthy controls. Between June 2010 and June 2011, the patients were prospectively diagnosed within 24-hour duration of symptom onset. After receiving detailed histories, all patients underwent detailed neurological examinations by the same neurologist. Patients with haemorrhagic stroke or with any other neurological diseases, or those who iron or anti-oxidant administered during the preceding month were excluded from the study. All patients underwent routine biochemical tests. The age, gender, hyperlipidaemia, diabetes mellitus, history of stroke or heart disease, smoking, and anti-hypertensive or anti-diabetic medications were recorded for all patients. Computerised tomography and/or cranial magnetic resonance imaging (MRI) scans, transthoracic echocardiography, and carotid and vertebral artery Doppler ultrasonography (USG) were also performed. Patients were classified according to the Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria. The National Institutes of Health Stroke Scale (NIHSS) was calculated for each patient. Those with diabetes mellitus or chronic kidney disease, and those using vasodilators containing nitrate or releasing nitrate as a metabolic product were excluded from the study. Blood samples were obtained within the first 24 hours of the onset of the stroke as far as the cases were concerned. The study

design was approved by the Ethics Committee of the Medical Faculty, University of Dicle, Turkey, and informed consent was obtained from all the subjects.

Serum samples were separated and stored at -20°C for 24 hours, and then transferred to -50°C until the time of the assay. SOD and PON1 activity was measured in accordance with literature.<sup>15,16</sup> Lipid peroxidation level of the serum was expressed as MDA and was measured as per literature.<sup>17</sup> Nitric oxide (NO) levels and TAC levels were also evaluated in line with literature<sup>18,19</sup> according to which the TAC assay is calibrated with hydrogen peroxide, and the results are expressed in terms of nmol H<sub>2</sub>O<sub>2</sub> equivalent/mg protein.<sup>20</sup>

All statistical analyses were performed using SPSS 11.5. Data was expressed as mean ± standard deviation. The normality of the distribution for all variables was assessed by the Kolmogorov-Smirnov test. Student's t-test was used for normally distributed variables and Mann-Whitney U-test was used for non-parametric variables. A p value less than 0.05 was considered statistically significant.

### Results

The 53 cases had 27 (51%) males and 26 (49%) females with a mean age of 64.5±15.8 years. The 40 healthy volunteers had 19 (48%) males and 21 (52%) females with a mean age of 66.3±13.9 years (Table-1). There was no statistically significant difference between the cases and the controls in terms of age (p>0.05) and gender distribution (p>0.05). Stroke risk factors, obesity and hypertension were evaluated in the cases and the controls. Obesity in the patients 15 (28.3%) and controls 13 (37.5%) was insignificant (p>0.05). Hypertension in cases 30 (56.6%) and controls 23 (57.5%) was also insignificant (p>0.05). The biochemical TAC, MDA, NO, PON1 and SOD results of the cases and the controls were also compared (Table-2). In patients with acute ischaemia, the MDA (147.3±59.3 vs. 112.4±28.5 nmol/gr protein, p<0.001), and SOD (4.40±0.79 vs. 3.35±0.51; p<0.001) concentrations were significantly higher than the controls. On the other hand, the NO (10.8±7.1 vs. 17.5±2.4 µmol/gr protein, p<0.001), PON1 (23.2±23.7 vs. 64.7±52.6; p<0.001) and TAC (0.77±0.38 vs. 0.95±0.30, p<0.015) concentrations were found to be significantly lower in the patients compared to the controls. A significant negative correlation was found between the

Table-1: Characteristics of stroke patients and control groups.

	Stroke patients (n=53)	Control (n=40)	P-value	95 % confidence interval
Mean age (years)	64.5 ± 15.8	66.3 ± 13.9	<0.57	0.99 (0.97-1.02)
<b>Gender</b>				
(Male / Female)	27 (51%) / 26 (49%)	19 (48%) / 21 (52%)	<0.88	0.94 (0.41-2.14)
Obesity	15 (28.3%)	13 (37.5%)	<0.66	1.22 (0.50-2.98)
Hypertension	30 (56.6%)	23 (57.5%)	<0.93	1.04 (0.45-2.38)

Table-2: Serum levels of malondyaldehyde (MDA), total antioxidant status (TAC), nitric oxide (NO), and activities of superoxide dismutase (SOD) and paraoxanase (PON1) in stroke patients and controls.

	Stroke patients (n=53)	Controls (n=40)	P-value	95 % confidence interval
MDA, (nmol/gr protein)	147.3±59.3	112.4±28.5	≤ 0.001	1.02 (1.01-1.03)
TAC, (mmol Trolox Eq./g protein)	0.77±0.38	0.95±0.30	≤ 0.015	0.23 (0.07-0.77)
NO, ( μmol/gr protein)	10.8±7.1	17.5±2.4	≤ 0.001	0.82 (0.75-0.89)
SOD, (U/L)	4.40±0.79	3.35±0.51	≤ 0.001	8.99 (3.79-21.32)
PON-1	23.2±23.7	64.7±52.6	≤ 0.001	0.97 (0.95-0.98)

NIHSS and TAC levels in the patients ( $p < 0.021$ ,  $r = -0.32$ ).

## Discussion

It was observed that during the acute phase, the MDA and SOD levels were increased in the serum of the patients with ischaemic stroke, while the NO, TAC and PON1 levels were decreased. In addition, a negative correlation was demonstrated between the TAC and the NIHSS, which is one of the scales indicating the severity of the stroke. These findings may indicate the importance of the anti-oxidant system in the pathogenesis and severity of the stroke.

Both experimental and clinical studies have shown that the production of free radicals causing oxidative stress plays an important role in the pathogenesis of the ischaemic brain damage which leads to functional losses in the brain subsequent to the stroke. There is strong evidence pointing out that the production of free radicals during the ischaemia and reperfusion is one of the important mechanisms causing brain damage. Due to certain reasons, the brain tissue is especially prone to the deleterious effects of the free radicals.<sup>21,22</sup> Free radicals and lipid peroxidation contribute to the physiopathology of the stroke. Serum MDA levels are widely accepted as markers of oxidative stress and lipid peroxidation. In various studies, the MDA levels were found to be increased in patients with acute stroke.<sup>8,23</sup> Moreover, correlations between the MDA levels, the severity of the stroke and the prognosis were observed in some studies.<sup>9,10</sup> In concordance with these studies, we also detected a marked increase in the MDA levels in ischaemic stroke patients. This finding may point to the occurrence of lipid peroxidation and cell damage in the acute phase of the ischaemic stroke. Studies where TAC is investigated together with the oxidant parameters in ischaemic stroke patients are limited in number.<sup>24,25</sup> Therefore, the antioxidant capacity that develops in the aftermath of an acute stroke may protect the brain against the negative effects of the free radicals produced during the ischaemia and reperfusion.<sup>26</sup> In a study, a significant relationship was observed between the cerebral ischaemic infarct volume, the degree of the neurological damage, and the level of the total plasma anti-oxidant activity. Another study also detected a negative correlation between the NIHSS and the anti-oxidant parameters.<sup>24</sup> In line

with our study, another study found TAC to be lower than the controls in 38 stroke patients.<sup>27</sup> In another study, the TAC levels were found to be lower in 31 acute ischaemic stroke patients compared to the controls.<sup>28</sup> Others observed that the TAC levels were decreased during the early phase (the first 24 hours) and that they were inversely proportional to the NIHSS scale.<sup>25</sup> Similar to earlier studies, we also found a negative correlation between TAC values and the NIHSS scale.<sup>24,28</sup> The distinctness of our study was the greater number of patients compared to the previous studies. The increase in the TAC levels in patients with ischaemic stroke may point to a depletion of the anti-oxidants during the acute phase of the stroke. For the purposes of our study, SOD and PON1 anti-oxidant enzymes were also tested in the serum besides the TAC levels. In line with two previous studies, we also observed lower PON1 levels compared to the controls.<sup>8,29</sup> Others detected higher serum MDA levels and lower anti-oxidant activity of PON1 in cases compared to the controls.<sup>8</sup> In our study, SOD levels in the patients were found to be lower than the controls. Anti-oxidants involving enzymatic systems like the SOD, transforming the superoxide anion into hydrogen peroxide, provide primary anti-oxidant defence. A study observed that SOD levels were increased in the cerebrospinal fluid (CSF)<sup>30</sup> of the patients with ischaemic stroke. The same study also suggested that the increased SOD activity in ischaemic brain damage is a good marker in CSF.<sup>30</sup> Similar to the previous studies, our study may also show that the increase in SOD may constitute an adaptive response to the oxidative stress in patients with ischaemic stroke.

NO leads to a relaxation in the smooth muscles of the blood vessels and plays a role in the regulation of the blood flow and blood pressure.<sup>31</sup> While there are studies pointing to an increase in the NO levels in patients with ischaemic stroke, other studies have reported decrease in the NO levels.<sup>6,23,32,33</sup> In our study, a significant decrease was detected in the NO levels within the first 24 hours in patients with stroke. A similar study comprising 49 patients, found NO levels to be lower than the controls.<sup>33</sup> Others have demonstrated a decrease in the NO levels in the plasma of the patients with ischaemic stroke, together with an increase in the peroxynitrite levels.<sup>32</sup> The decrease in the NO levels in our patients may be related

to the regulation of the cerebral blood circulation in acute cerebral ischaemia, or it may be metabolised during the NO peroxynitrite production due to oxidative stress.

## Conclusion

The study was able to demonstrate the role of oxidative stress in the pathogenesis of ischaemic stroke. Also, a negative correlation was observed between the TAC levels and the NIHSS scale. This finding supports the hypothesis that the antioxidant capacity has a beneficial effect on the clinical severity of the acute ischaemic stroke. Further studies may be helpful in providing larger scale information on the role of the oxidative stress in the pathophysiology of cerebral infarcts and to propose new agents for its treatment.

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