TOTAL OXIDATIVE STRESS, TOTAL ANTIOXIDANT STATUS AND ERYTHROCYTES STATUS IN PATIENTS WITH ACUTE ISCHEMIC STROKE

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ABSTRACT

Objectives: In this prospective study, we attempted to determine the initial oxidative-antioxidative status using a new automated measurement method. Serum total oxidant status (TOS), total antioxidant status (TAS), oxidative stress index (OSI) levels and erythrocyte status in patients with acute ischemic stroke (AIS) were detect and compared to those in controls.

Methods: In total, 51 patients with AIS and 49 sex- and -age matched healthy participant as controls were enrolled the study. With the new automated extent practice developed by Erel, Serum TOS, TAS and OSI was measured. In addition serum erythrocytes level were measured.

Results: Subjects suffering AIS had higher TOS and OSI level than those control subjects. In contrast the TAS level were moderate higher in the controls than patients. In addition serum Mean corpuscular volume (MCV) and Red cell distribution width (RDW) levels were higher in patients with AIS in accordance with the controls.

Conclusions: Patients with AIS are subjected to oxidative stress and thus, erythrocyte indices have a significant impact on this type of patients. However, further studies are needed to confirm this relationship.

Keywords: Total Oxidative stress, Total antioxidant status, Erythrocytes indices, Acute ischemic stroke, Emergency department.

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Introduction

Acute Ischemic Stroke (AIS) is defined as brain injury due to disruption of blood circulation. AIS is the consequence of non attendance of cerebral arterial blood supply, which leads to oxygen and glucose deficiency of the ischemic tissue. When cerebral arterial blood flow is not renovated within a short time, cerebral ischemia is typically occurring, with following neuronal dying within the perfusion area of the affected vessels. AIS is defined by a combined sequence of condition that develop over hours or even days\(^{1-3}\). Cerebral ischemia occurs when blood flow to the brain reduces to a level where the metabolic needs of the tissue are not met. Every bit conditions, a stroke causes dysfunction and death of neurons and neurological depredation that reflects the location and extent of the affected brain area\(^{12}\). Furthermore, the high lipid extent of the brain can react with reactive chemical oxygen production to produce radical oxygen product that lead to neuron membrane lipid oxidation\(^4\). These factors, named as radical oxygen products, are able to determine oxidative damage in brain tissue\(^5\). Total Oxidative Status is usually defined as a derangement between formation of free radicals and antioxidant defense system. Studies have demonstrated that detrimental oxidative products achieve their peak within the first 24-h following ischemic brain damage and that following brain damage may be the cause of death or the gravity of ischemia in patients with ischemic stroke\(^6\).
There are controversial opinion in clinical reports on oxidative stress in patients with AIS. In this prospective study, we attempted to determine the initial oxidative-antioxidative status using a new automated measurement method. Serum total oxidant status (TOS), total antioxidant status (TAS), oxidative stress index (OSI) levels and erythrocytes status in patients with AIS were detect and compared to those in controls.

Materials and methods

Fifty-one patients with AIS, who were admitted to the Department of Emergency medicine of the Affiliated Harran University Hospital (Sanliurfa, Turkey) from June 2012 to November 2012 were enrolled in the study. Additionally, we enrolled 49 sex - and -age matched healthy participant as controls. The subjects comprised 41 males and 59 females; mean age was 68.39±11.83 (range, 31-93 years) in the AIS patients and mean age was 65±9.95 years (range, 47-91 years) in the control subjects. If required, patient’ basic and advanced life support were given during admittance to emergency department. Vital functions were monitored and written informed consent was provided from the patients' legal administrator. The healthy subjects selected as controls, were informed about the study protocol, and written consent was obtained. Inclusion criteria were: age over 40 years, AIS within the first 24-h admitted to ED. Normal healthy controls were subjects with no acute ischemic stroke.

The study protocol was ruled in conformity with the 1989 Declaration of Helsinki and was authorized by the Ethics Committee of Harran University, Faculty of Medicine (Date:18.05.2012, No:24).

Exclusion criteria

To research the reserved effects of AIS on oxidative status, subjects with state of affairs that may have potentially affected TOS, TAS and erythrocytes status, such as chronic medical disorders (i.e., congestive heart failure, Hemolytic anemia and other hematological disorders, diabetes mellitus, chronic renal failure, or malignancy) or concomitant acute injuries (i.e. fall due to stroke), were removed from the study. None of the subjects were taking drugs known to affect oxidative status. In addition, patients who were taking anabolic drugs, diuretics, vitamins, or other antioxidants were excluded.

Blood sample collection

Venous blood specimens of 5 ml from patients and controls were withdrawn, accumulated in heparinizied tubes, and directly lay in ice at 4 °C. Blood samples of the patients were taken immediately after arrival at the emergency department (within 24 hours of injury).

The serum was wide apart from the cells by centrifugation at 4,000 rpm (Hettich® Universal 30 RF) for 5 minutes. Plasma specimens were stored at -80 °C (New Brunswick Scientifi®.C54285 model) until analysis.

Measurement of total oxidant status

With the new automated extent practice developed by Erel, Serum TOS was measured (9). Oxidants available in the specimen oxidize the ferror ion-o-dianisidine complex into ferric ions. The oxidation reaction is augmented by glycerol, which is plentiful in the reaction moderate. The ferric ions supply a colored complex with xylene orange in an acidic moderate. The color density, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. This analysis is adjusted with hydrogen peroxide, and the results are expressed in terms of micromolar hydrogen peroxide equivalent per litre (μmol H2O2 equiv/L). The analysis has superb sen-sitive amount of <2%.

Measurement of total antioxidant status

The TAS in serum was resolved using an auto-mated extent practice (10). Hydroxyl radicals, which are within the efficient biological radicals are made by the method. Radicals which produced by hydroxyl cation such as brown colored dianisidine are also potent radicals. Color formation is increased due to the oxidation reactions progress among dianisidinyl radicals and developing oxidation reactions. The oxidation reactions and color formation are suppressed by antioxidants in the model. Handling this assay, the antioxidative influence of the sample counter the potent free-radical reactions, which are initiated by the manufacturer hydroxyl radicals, was measured. TAS levels are expressed as mmolTrolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) equivalent/L. The sensitivity of the analysis was <3%.

Determination of the oxidative stress index

The OSI was estimated with respect to the behind formula: OSI (arbitrary units)=TOS (μmol
\( H_2O_2 \) equiv/L) / TAS (mmol Trolox, equiv/L) x 10-1.

**Other parameters**

Triglycerides (TG), high density lipoprotein (HDL) and low-density lipoprotein-cholesterol (LDL-C) levels were resolved using commercially present test kits (Abbott) with an autoanalyzer (Abbott Aeroset®, USA).

**Statistical analyses**

All statistical analyses were carried out using statistical analysis software (SPSS) type 20.0 (SPSS, Inc., Chicago, IL, USA). Categorical data are reported as frequencies and percentages; Student t test were performed using these categorical factors. Numerical data (e.g., oxidative and antioxidative factors; TOS, TAS, OSI and erythrocytes indices) are expressed as means and standard deviations. A p value <0.05 was considered as statistically significant.

**Results**

In table 1 are summarized demographic and clinical characteristics of the patients.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>AIS Group</th>
<th>Control Group</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/Female</td>
<td>21/30</td>
<td>20/29</td>
<td>0.651</td>
</tr>
<tr>
<td>Age (years)</td>
<td>68.39±11.83</td>
<td>65±9.95</td>
<td>0.397</td>
</tr>
<tr>
<td>DM</td>
<td>16 (32%)</td>
<td>15 (31.25%)</td>
<td>0.682</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>124.31±13.67</td>
<td>123.98±13.69</td>
<td>0.903</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>78.76±9.93</td>
<td>79.83±9.82</td>
<td>0.589</td>
</tr>
<tr>
<td>HR (times/min)</td>
<td>72.63±6.25</td>
<td>73.84±7.19</td>
<td>0.709</td>
</tr>
</tbody>
</table>

Data presented as mean±SD (standard deviation) of patients, * Student t test. Chi-square test. DBP - diastolic blood pressure, DM - diabetes mellitus, HR - heart rate, SBP - systolic blood pressure.

A total of 100 subjects were included in the study; 51 patients with AIS and 49 as controls. Of the 51 AIS patients 30 (58.8%) were female and 21(41.2%) male and of the 49 control subjects, 29 (59.2%) were females and 20 (40.8%) males. The average age of the patients was 68.39±11.83 years, and the average age of the controls was 65±9.95. Nevertheless with respect to gender and age there were no statistically significant differences between the patients with AIS and controls (p=0.651; p=0.397, respectively).

Similarly, no statistical difference was proved in systolic blood pressure (SBP) (p=0. 903), diastolic blood pressure (DBP) (p= 0.589), diabetes mellitus (DM) (p=0.682), and heart rate (HR) (p=0.709) between groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>AIS Group</th>
<th>Control Group</th>
<th>*P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLT,10^3 μL−1</td>
<td>259.07±66.36</td>
<td>265.52±43.24</td>
<td>0.568</td>
</tr>
<tr>
<td>PCT, %</td>
<td>0.23±0.04</td>
<td>0.22±0.03</td>
<td>0.604</td>
</tr>
<tr>
<td>PDW, fL</td>
<td>8.15±1.34</td>
<td>7.74±1.17</td>
<td>0.109</td>
</tr>
<tr>
<td>Glucose,mg/dl</td>
<td>148.99±71.69</td>
<td>128.27±55.88</td>
<td>0.110</td>
</tr>
<tr>
<td>LDL-C</td>
<td>126.27±27.83</td>
<td>113.93±31.15</td>
<td>0.045</td>
</tr>
<tr>
<td>TG</td>
<td>157.07±61.74</td>
<td>150.62±86.27</td>
<td>0.696</td>
</tr>
<tr>
<td>APTT</td>
<td>28.11±4.83</td>
<td>26.12±6.07</td>
<td>2.35</td>
</tr>
</tbody>
</table>

Table 2: Laboratory characteristics in patients with AIS and healthy controls*. APTT:activated partial thromboplastin time, LDL-C:low-density lipoprotein-cholesterol, PCT: plateletcrit, PDW: width of platelets in volume index, PLT:Platelet, TG: triglycerides. Data presented as mean±SD (standard deviation) of patients* Student t test.

As shown in the table 2 the mean serum LDL-C levels were higher in the patients with AIS than in the control group (126,27± 27.83 and 113.93±31.15, respectively). In addition the mean serum activated partial thromboplastin time (APTT) level was 28.11± 4.83 in the patients and 26.12±6.07 2.35 in controls. There were statistically considerable distinction between the patients with AIS and controls with respect to LDL-C levels (p=0, 045).
Nevertheless patients with AIS were not differ from those controls in Platelet (PLT), (p=0.568); plateletcrit (PCT) (p= 0.604), platelet distribution width(PDW) (p=0.109), Glucose (p=0.110), APTT (p=0.075) and TG (p=0.696).

As shown in the table 3 compared with healthy controls, subjects suffering from AIS had higher TOS (1.03±0.36 and 0.80±0.29; p=0.001, respectively) and OSI (3.57±1.50 and 2.86±1.08; p=0.007, respectively) level than those control subjects (Figure 1 and 2).

In contrast, as shown in figure 3, the TAS level were moderate higher in the controls than patients (28.09±6.16 and 25.56±6.36; p 0.046).

**Table 3**: TAS, TOS and OSI levels in patients with AIS and healthy controls*. 

<table>
<thead>
<tr>
<th>Parameters</th>
<th>AIS group</th>
<th>Control group</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAS, mmol Trolox eqv/L</td>
<td>25.56±6.36</td>
<td>28.09±6.16</td>
<td>0.046</td>
</tr>
<tr>
<td>TOS, mmol H₂O₂ Eqv/L</td>
<td>1.03±0.36</td>
<td>0.80±0.29</td>
<td>0.001</td>
</tr>
<tr>
<td>OSI, arbitrary unit</td>
<td>3.57±1.50</td>
<td>2.86±1.08</td>
<td>0.007</td>
</tr>
</tbody>
</table>

*Oxidative stress index, TAS:total anti anti oxidant status, TOS:total oxidative stress
*Data presented as mean±SD (standard deviation) of patients,* Student t test, Chi-square test.

**Fig. 1**: TOS level in patients with AIS and healthy controls.

**Fig. 2**: OSI level in patients with AIS and healthy controls.

**Fig. 3**: TAS level in patients with AIS and healthy controls.

**Table 4**: Erythrocytes status in patients with AIS and Controls*.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>AIS group</th>
<th>Control group</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC, 10⁶µl⁻¹</td>
<td>4.62±0.67</td>
<td>4.78±0.73</td>
<td>0.834</td>
</tr>
<tr>
<td>HGB, gr.dL⁻¹</td>
<td>13.99±2.33</td>
<td>13.40±1.76</td>
<td>0.315</td>
</tr>
<tr>
<td>MCH, pg</td>
<td>28.53±3.03</td>
<td>28.09±2.39</td>
<td>0.420</td>
</tr>
<tr>
<td>MCHC, g.dL⁻¹</td>
<td>31.16±1.53</td>
<td>31.64±1.00</td>
<td>0.071</td>
</tr>
<tr>
<td>RDW, per cent</td>
<td>14.21±3.09</td>
<td>13.10±1.48</td>
<td>0.024</td>
</tr>
</tbody>
</table>

* Data presented as mean±SD (standard deviation) of patients, * Student t test, Chi-square test. HCT: hematocrit, HGB:hemoglobin, MCH: mean corpuscular hemoglobin, MCV: mean corpuscular volume, RBC: red blood cell count, RDW: red blood cell distribution width.
As shown in the table 4 on the admission to the emergency department, of erythrocytes incience, serum MCV and RDW levels were higher in patients with AIS in accordance with the controls (p= 0.043; p=0.024, respectively). However, there were no significant differences in the other erythrocytes parameters (table 4).

Discussion

Our study simultaneously measured oxidant and antioxidant capacity in patients with AIS to evaluate oxidative stress using a recently developed automated assay, which have the most prognostic value in these patients.

In present study we also measured MCV and RDW levels in patients with AIS. The first findings of the study are: the oxidative stress parameter (TOS, OSI) of the AIS group was significantly greater than that in the control group, while total antioxidant parameters (TAS) of the AIS group were significantly lower than those in the control group. The second findings of the study are: MCV and RDW levels were significantly increased in patients with AIS. Thus we demonstrated the potential role of erythrocytes status and OS for early outcome of the patients with AIS in ED. Demographic characteristics for the AIS patients and controls were similar in our study. There were no statistically significant differences between the two groups with regard to age, gender, and the rates of DM, SBP, DBP and HR.

Ischemic stroke is result of the interruption or severe decrease of blood flux in cerebral arteries with restriction in the delivery of basic nutrients to a cerebral region\(^{(11-12)}\). Stroke remains the third most common reason to death, especially in the elderly and its mortality rate in the acute phase is as high as 20%\(^{(13)}\).

It is reputed that oxidative stress contributes to progress of stroke via different mechanisms, such as excitotoxicity resulting in cellular enzymes activation\(^{(14)}\). TOS, as an early oxidative stress biomarker, may reflect severity of injury and provide an early indication of outcome in patients with AIS. In the present study patients had a higher TOS and OSI levels. In contrast, the TAS level were moderate higher in the controls than patients with AIS. Several studies have investigated effects of oxidative stress in patients with AIS\(^{(15-17)}\). Endogenous antioxidant capacity can be overwhelmed, leading to raised superoxide and hydrogen peroxide concentrations, though raised evidence of oxidant spaces can be formed in reply to ischemia\(^{(18)}\). While reduced catalase and SOD activities in AIS patients, reported in a study increased plasma MDA levels and GSH-Px activity\(^{(17)}\). Regarding the effects of SOD enzyme efficiency as an antioxidant in AIS, previously Controversial Reports has been shown. Cherubini et al. and Demirkaya et al. found significantly reduced SOD activity in AIS\(^{(19-20)}\).

On the contrary, Kossi and Zakharyan reported a significant difference in the serum SOD activity in AIS patients and controls\(^{(20)}\). These conflicting results may be produced by the presence of different SOD isoforms and variations in the process used to measure SOD activity.

Brain injury is intervened by ROS, including very transient substances such as nitric oxide, hydroxyl radicals, hydrogen peroxide, peroxynitrite and superoxide anions\(^{(21-22)}\). The brain is especially sensitive to oxidative injury because of its high polyunsaturated fatty acid content and proportionately large share of total body oxygen consumption\(^{(23)}\). Approach to detect oxidative stress in previous studies have been limited to measurements of single parameters, such as antioxidant concentrations and lipid peroxidation levels\(^{(24-26)}\). Though they may not provide the clinician with a complete assessment of the degree of oxidative stress occurring in a patient with AIS, these parameters may be helpful. But this beneficial results have a limitation in clinical practices. We used a new automated process to define oxidative stress in this study.

Compared to available methods this method has several advantages such as simple and cheap and it can easily be fully automated. In addition, it is dependable and susceptible and does not interact with occurring serum components such as bilirubin, serum lipids, and anticoagulants\(^{(27)}\).

In our previous study, we reported that assessed measures of TOS and OSI in the children with head injury and demonstrated a reduction in TAS compared to controls\(^{(27)}\).

Similarly in our present study we observed significant increase in TOS and OSI activity in the patients with AIS following 24-h period. In contrast serum TAS activity were moderate higher in the healthy controls than patients with AIS. These data indicate that the plasma TOS and OSI levels of the patients with AIS in the present study had prognostic value during the early period. Thus probably it may provide an early assessment of patients in the emergency department.
In the present study MCV and RDW levels were higher in AIS patients in accordance with the controls. RDW is suggested to be a biomarker reflecting a proinflammatory condition. Oxidative stress and inflammation increase RDW and reducing red cell life span\(^{18,29}\).

Previously there are only a few study on the relation between RDW and cardiovascular diseases. Skjelbakken et al. reported a strong association between RDW and myocardial infarction among smokers\(^{30}\). Furthermore, we have not seen any report related to MCV and RDW in acute ischemic stroke in literature. Although the increase in both parameters, there weren’t positive correlations in oxidative stress parameters with MCV and RDW in our study. Perhaps this is the most important deficiency in our study.

As a consequence, a increased in TOS activities despite decreased TAS activities were observed in patients experiencing AIS. Patients are subjected to oxidative stress and thus, erythrocytes indices have a significant impact on this type of patients. However, further studies are needed to confirm this relationship.

**Limitations**

The study has several possible limitations; first, the present study was performed in a single department, and we were able to provide data containing only one-time point measurement parameters from each patient. Second, there was a relatively small number of AIS patients. Third, our study was limited to assessing in-ED outcomes, and did not assess differences over longer periods.

**References**


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