Total Antioxidant Capacity Predicts Outcome in Acute Ischemic Stroke Subtypes in Egyptian Patients

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Background: Oxidative stress after ischemic stroke contributes to neuronal cell injury. We tried to demonstrate an association between total antioxidant capacity (TAC) levels and outcomes after acute ischemic stroke (AIS). *Methods:* We enrolled 60 patients (36 females and 24 males) who were admitted to our hospital due to AIS, in addition to 30 age and sex-matched healthy controls. TAC levels were measured on day 1 of stroke onset, the relationships between TAC levels, stroke subtypes, and clinical outcomes based on the National Institutes of Health Stroke Scale and modified Rankin scale upon discharge were evaluated. *Results:* TAC levels were significantly lower in AIS patients than control ($P < .001$) being much lower in patients with large-vessel cerebral infarction than in those with small-vessel infarction. We investigated whether TAC concentrations reflected the severity and outcome of ischemic stroke and we found a significantly lower concentration of TAC in the poor outcome group than in the good outcome group ($P < .001$). *Conclusions:* Our findings suggested that the biochemical changes related to TAC and oxidative stress may be considered a marker of ischemic brain injury and clinical outcome of ischemic stroke.

Key Words: TAC—acute ischemic stroke—stroke subtypes—clinical outcome

Introduction

Acute ischemic stroke (AIS) is a common cause of permanent disability in adults worldwide. Ischemia leads to increased production of free radicals and reactive oxygen species (ROS) in tissue and plasma through several mechanisms.[1] In healthy individuals, antioxidant activity counterbalances free radical production, but in the case of ischemia, the balance between ROS and antioxidant activity is shifted toward free radicals causing oxidative stress that potentially leads to cellular lipids, proteins, and DNA damage.[2] Oxidative stress is a pivotal event in the setting of AIS and may contribute to stroke outcome.[3,4]

The antioxidant defense system has been studied in stroke patients with regard to enzymes, including superoxide dismutase and glutathione peroxidase[5,6] and nonenzymatic antioxidants such as ascorbic acid, α-tocopherol, carotenoids, and uric acid[7-9]; however, total antioxidant capacity (TAC) measurement provides better indicator of antioxidant activity rather than individual antioxidants levels.[10]

Circulating TAC in AIS patients and its relation to stroke subtypes and outcome has been scarcely studied, and the findings are conflicting. In some studies, circulating TAC levels in AIS patients were lower than in healthy control subjects,[9,7,11] while in another study no differences were found.[12] Finally, higher circulating TAC was reported by Lorente et al.[13] Only 1 study tried to find the relation between TAC and stroke subtypes.[14] Thus, the objective of this study was to determine whether there is an association between circulating TAC levels, stroke subtypes, and outcome in patients with AIS.

Subjects and Methods

Sixty patients with AIS were enrolled in this study in the Department of Neurology, Zagazig University Hospital, from November 2016 to June 2017. Diagnosis was based on clinical presentation, neurologic examination, and results of brain imaging either computed tomography (CT) or magnetic resonance imaging (MRI) with diffusion-weighted imaging. Patients aged 18-80 years with acute

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non−cardioembolic ischemic stroke were included and divided into 2 major etiologic subtypes, that is, large-vessel disease (LVD) and small-vessel disease (SVD) according to the TOAST (Trial of Org 10172 in Acute Stroke Treatment) classification.[15] National Institutes of Health Stroke Scale (NIHSS) was assigned both at admission and upon discharge to determine neurological deficits. The modified Rankin scale (mRS) was also used to assess their clinical outcomes at discharge.[16] Good outcome was defined as a 3-month mRS of 0-2 without any cardiovascular event. Poor outcome was defined as mRS of 3-6. Conventional vascular risk factors including age, hypertension, diabetes mellitus (DM), hyperlipidemia, smoking, body mass index (BMI), and previous stroke were evaluated. Patients whose data could not be evaluated at the time of stroke onset and those with hemorrhagic infarction were excluded from this study. Patients with cardioembolic stroke, other determined causes and undetermined causes of stroke, and those with underlying neoplasm, end-stage renal disease, liver cirrhosis, and congestive heart failure, patients taking vitamins or other antioxidants were excluded. Clinical examination, electrocardiography, and cardiac echo ultrasound were conducted to exclude cardioembolic stroke. Patients with fever or any infectious disorder were also excluded. For comparison, 30 subjects who are age- and sex matched with no known history of stroke, cardiovascular disease, DM, and any other vascular risk factor or metabolic disease were enrolled as the control group. The study was approved by the local ethics committee, and all patients gave their informed consent to participate in the study.

MRI was done at MR Unit of Radiology Department of Faculty of Medicine, Zagazig University Hospitals using a German Achieva MRI scanner, Philips 1.5 tesla (T1, T2, and flair). MRI sequences at slice thickness 5 mm (using MR head coil) were done. CT using GE ProSpeed Dual slice FII CT with MX 135 tube multidirector machine (Ingenuity, Philips, Veensluis, the Netherland) was used.

Blood samples were collected in tubes on the first day of stroke onset. We allowed these samples to coagulate during 10 minutes at room temperature then we obtained serum samples by centrifugation at $1000 \times g$ for 15 minutes. Then, the serum samples were aliquoted and frozen, until processing, at −80°C.

TAC in serum samples were assayed using antioxidant assay kit (Cayman Chemical Corporation, Ann Arbor, MI). The assay depends on the capacity of antioxidants in the serum sample to inhibit the oxidation of ABTS (2,2′-azino-di-3-ethylbenzothiazoline sulfonate) to ABTS by metmyoglobin. The ability of the antioxidants in the sample to prevent ABTS oxidation is compared with that of Trolox, a water-soluble tocopherol analogue, and TAC is measured as molar Trolox equivalents. All samples were measured in duplicate at 20-fold dilutions in assay buffer following manufacturer's instructions (PerkinElmer, Waltham, MA). The serum concentration of TAC was expressed in mmol/L. Serum TAC processing was performed by a laboratory technician blinded to all clinical data. TAC assay was performed in the laboratory of the Zagazig University Hospitals (Sharkia, Egypt).

Serum TAC assay, used in present study, is a reliable and easy method. In this protocol, lipid soluble and water-soluble antioxidants are not separated, thus the combined antioxidant activities of all its components, including glutathione, uric acid, vitamins, lipids, and proteins, were assessed.

Statistical Analysis

All statistical analyses were performed using SPSS type 20.0 (SPSS, Inc, Chicago, IL) software for Windows. Data were expressed as mean ± standard deviation, and categorical variables were compared by using a χ^2 test. Student's t test for unpaired data was used to compare TAC concentrations of patients and controls, patients with LVD and SVD and in addition, analyses of the differences in TAC levels stroke patients with good and poor outcome. P values $<.05$ were considered statistically significant. Regarding the variables correlating with TAC, the association was tested using multiple regression analysis of covariance to adjust for the confounding variables affecting the association. Possible confounds included age, hypertension, smoking, dyslipidemia, previous stroke history, and number of risk factors.

Results

In Table 1, we summarized demographic and clinical characteristics of the study subjects. A total of 90 subjects were included in the study: 60 patients with AIS and 30 healthy controls. Of the 60 AIS patients, 36 (60%) were females and 24 (40%) were males, and of the 30 control subjects, 19 (63.3%) females and 11 (36.6%) males. There was no difference among the 2 groups regarding age and gender, while systolic and diastolic blood pressure, dyslipidemia, and smoking were significantly higher in stroke patients than in control subjects ($P < .001$ and $P < .03$, respectively). TAC levels were significantly lower in AIS patients than control.

Regarding stroke subtypes in our patients, 34 (56.6%) had LVD, while 26 (43.3%) had SVD. As for vascular risk factors, prevalence of hypertension was the highest in patients with SVD, and incidence of DM and dyslipidemia were higher in those with LVD infarctions. The number of vascular risk factors was also higher in LVD infarction compared to SVD group. The LVD group had higher baseline NIHSS and higher mRS (Table 2). Furthermore, the current study demonstrates that TAC levels are much lower in patients with large-vessel cerebral infarction than in those with small-vessel infarction (Fig 1).

When comparing patients with a poor outcome (mRS ≥3; 24 patients) and a good outcome (mRS ≤2; 36 patients), no significant differences were observed for age, history of previous stroke, while after further division of cases into age groups a significant difference becomes

Table 1. *Demographics, clinical data, and TAC concentrations of all participants*

	AIS N = 60	Control N = 30	t/χ^2	*P* value
Age (y) (mean ± SD)	68.39 ± 11.83	65 ± 9.95	1.35	.18 NS
Sex (F/M)	36/24	19/11	0.09	.76 NS
SBP (mm Hg)	157.5 ± 24	115.6 ± 23.2	7.89	<.001 HS
DBP (mm Hg)	91.5 ± 15	74.7 ± 25.5	3.93	<.001 HS
BMI	25.5 ± 3.3	22.3 ± 2.4	4.72	<.001 HS
Dyslipidemia (%)	35 (58.3%)	10 (33.3%)	5	.03 S
Smoking (%)	33 (55%)	9 (30%)	5.02	.03 S
Previous stroke (%)	18 (30%)	0 (0%)	11.25	<.001 HS
TAC (mmol/L)	1.7 ± 0.30	4.20 ± 0.50	29.59	<.001 HS

Abbreviations: AIS, acute ischemic stroke; BMI, body mass index; DBP, diastolic blood pressure; HS, highly significant; NS, nonsignificant; SBP, systolic blood pressure; Sig, significant; TAC, total antioxidant capacity; *t*, Student's *t* test; χ^2, chi-square test.

Table 2. *Characteristics of AIS patients recruited in this study*

	SVD N = 26	LVD N = 34	t/χ^2	*P* value
Age	69.1 ± 11.5	67.6 ± 8.8	.57	.57 NS
Sex (F/M)	16/10	20/14	.05	.83 NS
BMI	23.22 ± 4.2	25.12 ± 3.4	1.94	.06 NS
Hypertension, n (%)	26 (100%)	28 (82.4%)	5.09	.02 Sig
DM, n (%)	13 (50%)	19 (55.9%)	.20	.65 NS
Dyslipidemia, n (%)	14 (53.8%)	21 (61.8%)	4.85	.03 Sig
Smoking, n (%)	15 (57.7%)	18 (52.9%)	.13	.71 NS
Previous stroke, n (%)	7 (26.9%)	11 (32.3%)	.21	.65 NS
Number of risk factors	1.2 ± 0.1	1.9 ± .2	16.33	<.001 HS
Baseline NIHSS	5 ± 1.3	11 ± 2.2	12.34	<.001 HS
mRS at discharge	2.21 ± .42	1.24 ± .25	11.15	<.001 HS
TAC	2.51 ± .20	1.2 ± .13	30.68	<.001 HS

Abbreviations: BMI, body mass index; DM, diabetes mellitus; HS, highly significant; LVD, large-vessel disease; mRS, modified Rankin scale; NIHSS, National Institutes of Health Stroke Scale; NS, nonsignificant; Sig, significant; SVD, small-vessel disease; TAC, total antioxidant capacity; *t*, Student's *t* test; χ^2, chi-square test.

evident. Prevalence of risk factors for arteriosclerosis was significantly higher in patients with poor outcome (Table 3). Among patients with LVD infarctions, significantly more number of patients had a poor outcome; accordingly, NIHSS scores at admission were higher in patients with a poor outcome. We investigated whether TAC concentrations reflected the severity and outcome of ischemic stroke and we found a significantly lower concentrations of TAC in the poor outcome group than in the good outcome group on day 1 after stroke ($P < .001$; Fig 2). These findings suggested that the biochemical changes related to TAC and oxidative stress may be considered a marker of ischemic brain injury and clinical outcome of ischemic stroke.

Potential variables were analyzed using multiple logistic regression model (Table 4). Only the stroke subtype (odds ratio [OR]: 12.38, 95% confidence interval [CI]: 3.09-49.61; $P < .001$), NIHSS score (OR: 6.74, CI: 2.03-20.53; $P = .02$), mRS scale (OR: 4.76, 95% CI: 1.98-13.96; $P = .03$), and serum TAC on day 1 after stroke (OR: 3.29, 95% CI: 1.24-6.65; $P = .04$) were independently associated with 3-month outcome. The sensitivity of TAC at cutoff of 2.5 was 92.9%, specificity was 88.3%, and the accuracy was 91.1% (Table 5, Fig 3). There was a significant inverse

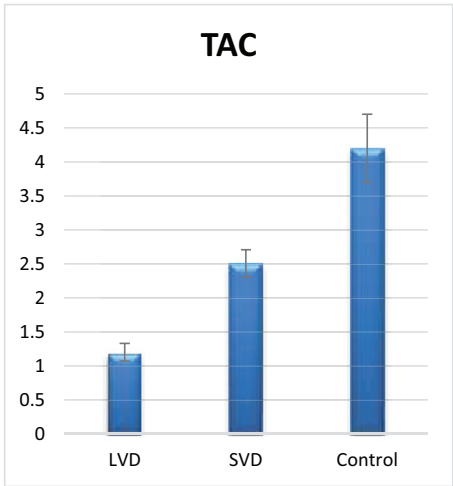

Figure 1. *Comparison of TAC levels in patients with SVD and LVD and control.*

Table 3. *Demographics, clinical data, and TAC concentrations of patients with good and poor outcomes*

	Good outcome (mRS ≤2) N = 36	Poor outcome (mRS ≥3) N = 24	t/χ^2	*P* value
Age	66.1 ± 11.5	68.6 ± 8.8	.90	.37 NS
Age groups				
18-40 y	12	3		
>40-60 y	14	7	6.25	.04 S
>60 y	10	14		
Sex (F/M)	19/15	15/9	0.25	.61 NS
Stroke subtype (SVD/LVD)	23/13	3/21	15.49	<.001 HS
BMI	24.21 ± 2.2	25.3 ± 2.3	1.85	.07 NS
Hypertension, n (%)	32 (88.9%)	22 (91.7%)	.12	.73 NS
DM, n (%)	17 (47.2%)	15 (62.5%)	1.35	.26 NS
Dyslipidemia, n (%)	22 (61.1%)	13 (54.2%)	.29	.59 NS
Smoking, n (%)	18 (50%)	15 (62.5%)	.90	.34 NS
Previous stroke, n (%)	10 (27.8%)	8 (33.3%)	.21	.65 NS
Number of risk factors	1.1	1.9	20.52	<.001 HS
Baseline NIHSS	5 ± 2.40	12.24 ± 4.1	9.81	<.001 HS
mRS at discharge	2.21 ± .42	4.24 ± .25	126	<.001 HS
TAC	2.60 ± .50	1.3 ± .25	11.77	<.001 HS

Abbreviations: BMI, body mass index; HS, highly significant; LVD, large-vessel disease; mRS, modified Rankin scale; NIHSS, National Institutes of Health Stroke Scale; NS, nonsignificant; Sig, significant; SVD, small-vessel disease; TAC, total antioxidant capacity; *t*, Student's *t* test; χ^2, chi-square test.

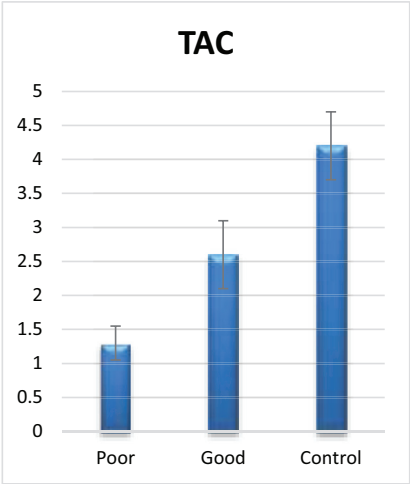

Figure 2. *Comparison of TAC levels in patients with good outcome and poor outcome and control.*

Table 4. *Multivariate analysis of significant factors for stroke outcome in the cases group*

Variables	OR	CI	*P*
Age	1.45	.64-3.84	.24 NS
Gender	2.10	.83-2.38	.39 NS
Stroke subtype	12.38	3.09-49.61	<.001 HS
Number of risk factors	1.76	.72-2.94	.28 NS
Baseline NIHSS	6.74	2.03-20.53	.02 Sig
mRS at discharge	4.76	1.98-13.96	.03 Sig
TAC	3.29	1.24-6.65	.04 Sig

Abbreviations: CI, confidence interval; mRS, modified Rankin scale; NIHSS, National Institutes of Health Stroke Scale; OR, odds ratio; TAC, total antioxidant capacity.

correlation between TAC levels and NIHSS scores (r = −.38, P = .02; Fig 4).

When we assessed the influence of different risk factors on TAC levels, DM, dyslipidemia, smoking and number of risk factors were significant (Table 6). With further multivariate analysis of TAC levels with these risk factors, DM and dyslipidemia significantly influenced TAC levels (Table 7).

Discussion

The brain is particularly susceptible to oxidative stress because it utilizes the highest amount of oxygen compared with other body organs. The brain also contains high concentrations of polyunsaturated fatty acids that are prone to lipid peroxidation, and is rich in iron, which can catalyze hydroxyl radical formation.[17] Approach to detect oxidative stress in previous studies has been limited to measurements of single parameters, such as individual enzymatic and nonenzymatic antioxidants or lipid peroxidation levels.[18-20] Though these parameters may be helpful, their beneficial results have a limitation in clinical practice as they may not provide the clinician with a complete assessment of the degree of oxidative stress occurring in a patient with AIS. So, we used TAC as a biomarker of the total antioxidant state in patients with AIS.

The present study has 4 major findings. First, patients with AIS in the acute phase had significantly lower TAC levels than the controls. Cerebral ischemia and tissue injury are associated with increased production of ROS, there may be rapid consumption of antioxidants in response to this oxidative stress leading to low TAC levels

Table 5. *Validity of TAC in prediction acute ischemic stroke*

Cutoff	AUC	CI	Sens.	Spec.	+PV	−PV	Accuracy	*P* value
<2.5	.92	.85-1.02	92.5	88.3	94.1	85.5	91.1	<.001*

Abbreviations: AUC, area under the curve; CI, confidence interval; PV, predictive value.
*Statistically significance.

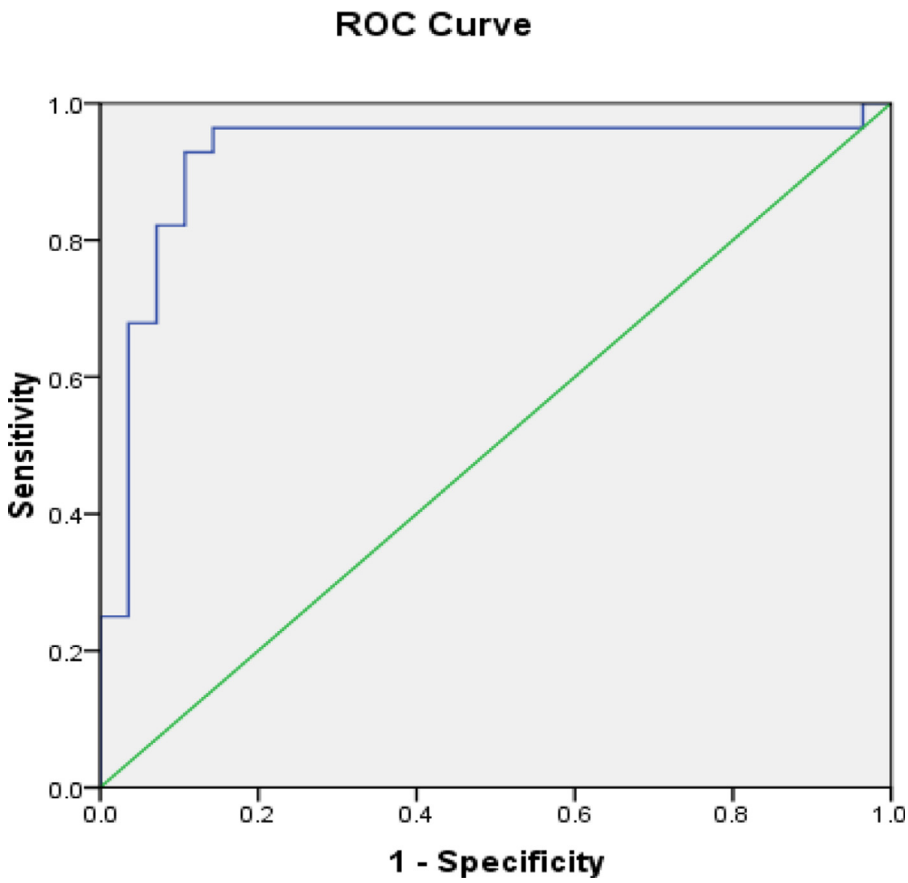

Figure 3. *ROC curve of TAC as a prognostic marker for acute ischemic stroke.*

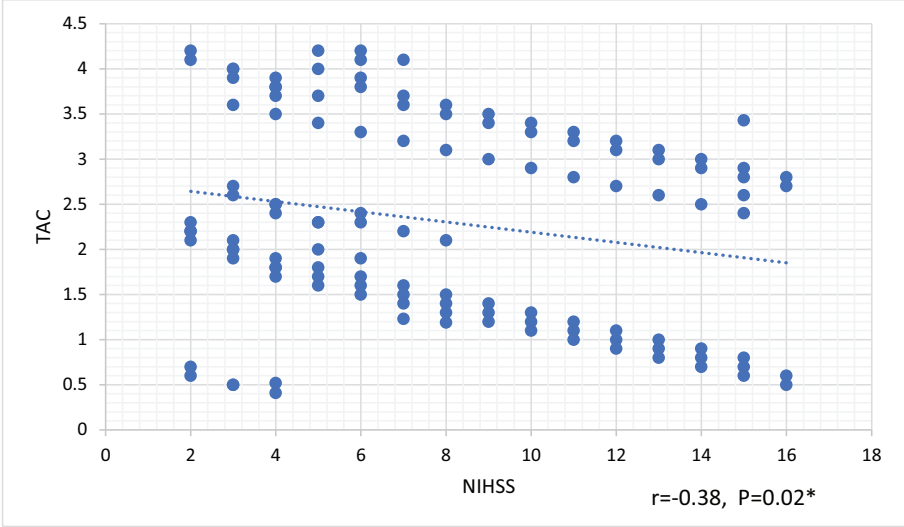

Figure 4. *Correlation between TAC and NIHSS among cases group.*

Table 6. *Relation between TAC level and different risk factor among the studied group*

	TAC >2 N = 23	TAC ≤2 N = 37	χ^2	*P* value
BMI >25 kg/m^2, n (%)	8 (34.7%)	11 (29.7%)	.16	.68 NS
Hypertension, n (%)	20 (86.9%)	34 (91.9%)	1.02	.31 NS
DM, n (%)	7 (30.4%)	24 (64.8%)	**6.73**	**.009 HS**
Dyslipidemia, n (%)	8 (34.7%)	27 (70.2%)	**8.51**	**.003 HS**
Smoking, n (%)	8 (34.7%)	23 (62.2%)	**4.26**	**.04 S**
Previous stroke, n (%)	7 (30.4%)	11 (29.7%)	.003	.95 NS
Number of risk factors >2, n (%)	11 (47.8%)	26 (70.2%)	**4.27**	.04

Abbreviations: BMI, body mass index; DM, diabetes mellitus; HS, highly significant; NS, nonsignificant; Sig, significant; TAC, total antioxidant capacity; χ^2, chi-square test.

Highly significant and significant values appearing in bold.

Table 7. *Multivariate analysis of significant factors for low TAC level among the studied group*

Variables	OR	CI	*P*
DM	3	1.39-6.49	.009 HS
Dyslipidemia	3.66	1.68-7.98	<.001 HS
Smoking	2.05	0.97-4.83	.07 NS
More than 2 risk factors	1.46	0.68-3.13	.32 NS

Abbreviations: CI, confidence interval; DM, diabetes mellitus; OR, odds ratio.

in patients with AIS compared to control. Some studies reported similar results to ours regarding lower circulating TAC levels in AIS patients than in healthy control subjects.[9,6,11,21] In contrast, Lorente et al[13] and Guldiken et al[14] reported higher levels of TAC in their ischemic stroke patients. According to them, TAC upregulation in patients with AIS might be a compensatory mechanism for higher ROS production in response to ischemia.

The second finding in this study is that the level of TAC is significantly lower in patients with LVD than in those with SVD. Oxidant/antioxidant imbalance is related to the different pathogenesis in the 2 major subtypes of noncardioembolic stroke and this may explain the differences in TAC levels between LVD and SVD infarctions. The pathogenesis of small-vessel infarction is lipohyalinosis,[22] while atherothrombosis is the major cause of large-vessel cerebral infarction.[23] Thus, different subtypes of ischemic stroke have different pathogenesis, with different consequences on oxidative stress. Infarct size can also influence oxidative stress with smaller size infracts like that of SVD produce lesser amount of oxidative stress.[24,25] In addition, patients with LVD infarction had more vascular risk factors for AIS than those with SVD, these vascular risk factors such as hypertension, DM, hyperlipidemia, and BMI influence TAC levels, even with increasing number of risk factors there are higher oxidative stress and lower TAC levels and this is consistent with the previous study of Nakajima et al.[25]

Third, the lower levels of TAC in the acute phase of AIS is associated with clinical stroke severity and poor outcome as evidenced by the significant negative correlation with NIHSS and mRS scores (ie, clinical outcomes at discharge) and these results are parallel to those of Gariballa et al.[21] Lastly, the most important finding in this study is that TAC level is an independent predictive factor of outcome according to the results of multiple logistic regression analysis. Accordingly, it can be a useful marker for prediction of outcome after ischemic stroke.

Despite evidence of deleterious contributions of oxidative stress in AIS, there remains doubt over the clinical efficacy of antioxidants in this setting.[26]

Conclusions

These results indicate that oxidative stress is increased and that the majority of antioxidants are reduced; this suggests the possibility of therapeutic intervention with antioxidant agents especially for the acute-phase (24 hours) treatment of stroke. Oxidative stress and TAC in the later periods of AIS need to be explored in further studies.

Limitations

Some limitations of this study should be mentioned. First, TAC should be interpreted together with oxidative stress markers not as a separate entity. Second, serial assessment of TAC at days 1 and 7 to compare changes in TAC levels in acute and subacute stages of stroke. Third, oxidative stress may be influenced by other drugs (eg, antiplatelet, angiotensin II type 1 receptor blockers, and hypoglycemic drugs). Since the use of these drugs depends on the preference of the attending physician, this may cause potential bias in statistical analysis and in drawing conclusions. Finally, our study consisted of small numbers of patients and we think larger studies are needed to assess oxidative stress in AIS patients.

Supplementary Materials

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.jstrokecerebrovasdis.2019.03.053.

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