Neurology Laboratory **Edited by: T. Weber/U.K. Zettl**

Ozlem Dogan*, Ucler Kisa, Ali Kemal Erdemoglu, Murat Kacmaz, Osman Caglayan and Huseyin Kurku

Oxidative and nitrosative stress in patients with ischemic stroke

https://doi.org/10.1515/labmed-2018-0036
Received April 5, 2018; accepted August 23, 2018; previously published online September 18, 2018

Abstract

Background: Oxidative and nitrosative stress is well believed to play a role in the pathogenesis of ischemic stroke. This study aims to evaluate the time course of oxidative and nitrosative stress in ischemic stroke.
Methods: In total, 27 healthy individuals, 22 individuals with high risk of ischemic stroke due to hypertension and diabetes mellitus, and 20 patients with acute ischemic stroke hospitalized at the Neurology Department of the Kırıkkale University School of Medicine were enrolled in the study. Venous blood was collected at admission (hour 0) and again at hours 24, 48, 72, and 96. Nitric oxide (NO), malondialdehyde (MDA), total oxidative stress (TOS), oxidative stress index (OSI), and total antioxidant status (TAS) were measured and compared among stroke patients and control groups.
Results: Blood NO was significantly higher in the patient group at 0, 24, 48, and 72 h compared to the healthy and high-risk control groups, and lower at 96 h than at early times within the patient group ($p < 0.001$). MDA was higher in patients than the healthy control group at all times. Conversely, TOS and OSI were significantly lower in the patient group than the healthy control group at 96 h and the high-risk control group at 72 and 96 h ($p < 0.05$). There was a significant correlation between initial NO (0 h) and duration of hospitalization ($r = 0.71$; $p = 0.0003$).
Conclusions: These findings suggest a substantial early increase in oxidative and nitrosative stress in ischemic stroke patients during the first 2 days post-admission. However, TOS was lower by days 3–4, likely due to pathological recovery and local/systemic defense systems. The correlation between elevated serum NO during the acute phase of stroke and duration of hospitalization suggests NO as a potentially valuable predictor of ensuing oxidative damage and clinical outcome.

Keywords: antioxidant status; ischemic stroke; malondialdehyde; nitric oxide; oxidative stress; total oxidative stress.

Introduction

Stroke is the most frequent cause of mortality and morbidity in developed countries. While stroke is a heterogeneous syndrome, studies have suggested that ischemic stroke accounts for 70%–80% of all stroke cases [1]. Brain ischemia is defined as cerebral hypoperfusion and is associated with cellular bioenergy deficiency, which leads to excessive formation of free radicals regardless of the source through damage to nucleic acids, lipids, proteins, and other biomolecules, e.g. polysaccharides [2].

Oxidative stress plays a major role in the physiopathology of stroke by inducing the generation of cytotoxic free oxygen radicals, and is an independent risk factor for poor outcome. During stroke, reactive oxygen oxidative stress develops in the case of increased steady-rate concentrations of species (ROS) and reactive nitrogen species (RNS) are produced both enzymatically and non-enzymatically. Oxidative stress develops when pro-oxidant activity exceeds endogenous antioxidant defense capacity [3].

Increased oxidative stress may affect nitric oxide (NO), which has an important role in the control of cerebral blood flow, thrombogenesis, and modulation of neuronal activity, and plays an important role in the pathogenesis of cerebral ischemic stroke. Increased NO concentrations associated with ischemia give peroxynitrite through enhanced biodegradation of NO by superoxide radical [4]. NO is produced by nitric oxide synthases (NOSs) neuronal NOS

***Correspondence: Ozlem Dogan,** MD, Ankara University of Medical School, Department of Biochemistry, Ankara, Turkey, Phone: 05327111968, E-Mail: ozlemceylandogan@gmail.com
Ucler Kisa, Murat Kacmaz and Osman Caglayan: Kırıkkale University of Medical School, Department of Biochemistry, Ankara, Turkey
Ali Kemal Erdemoglu: Kırıkkale University of Medical School, Department of Neurology, Ankara, Turkey
Huseyin Kurku: Konya Training and Research Hospital, Department of Biochemistry, Konya, Turkey

(nNOSs), inducible NOSs, and endothelial NOSs (eNOSs). The isoform of NOSs determines whether it functions as neuroprotective (eNOSs) or neurodestructive (nNOSs and iNOSs) [4, 5]. Irrespective of the type of NO, the effects of NO in ischemic stroke are controversial [5].

The brain is particularly vulnerable to the increases in ROS and RNS due to the lower neuronal antioxidant capacity, high concentrations of sensitive polyunsaturated membrane lipids, greater basal O_2 consumption, and higher levels of iron, which acts to catalyze the conversion of H_2O_2 to highly reactive hydroxyl radicals [6].

Nitro-oxidative stress is induced by excessive production of ROS, which is associated with increased synthesis of NO by inducible NOS (iNOS). The increased production of reactive species might be associated with a decrease in antioxidant capacity. This is why nitro-oxidative stress should be evaluated by measuring ROS (including lipid peroxides malondialdehyde [MDA]) NO, total oxidative stress (TOS), total antioxidant status (TAS) and oxidative stress index (OSI).

The present study compared blood MDA, NO, TOS, TAS, and OSI levels in ischemic stroke patients during the early acute period and through a 4-day period with increased cerebral edema with two different control groups (healthy and high stroke risk) to examine how oxidant levels and antioxidant systems are affected.

Materials and methods

The study was planned for 30 patients as well as 30 healthy and 30 pathological control subjects considering likely exclusions in the course of the study. Upon re-evaluation of the exclusion criteria, 20 patients, 27 healthy control subjects, and 22 pathological control subjects were involved in the study.

This prospective study enrolled a total of 20 patients without previous history of cerebrovascular disease, cerebral hemorrhage, transient ischemic attack, or stroke, who were diagnosed with ischemic stroke upon clinical and neuroradiological examination. All presented to the hospital within the first 24 h of rapid onset focal or global cerebral ischemic symptoms. The first blood sample was collected prior to commencement of any medical treatment (defined as hour 0). Additional blood samples were collected at hours 24, 48, 72, and 96. The present study did not involve any comparison of location, volume, or etiology of stroke.

Two different control groups were recruited; 27 healthy individuals were presented to the hospital for health check without previous stroke history or systemic diseases. Furthermore, the control group was not on any antioxidant vitamins and medicines and did not smoke or use alcohol, In total, 22 individuals were with elevated risk of ischemic stroke due to hypertension and diabetes mellitus. Patients with diabetes mellitus and hypertension, who were being followed-up at our hospital, were involved in the study. The pathological control group was involved in the study to differentiate whether the data collected from the patient group were associated with the stroke process or with such pathologies, e.g. diabetes mellitus and hypertension. Blood NO, MDA, TOS, and TAS were measured, and the OSI was calculated at each time point.

The study protocol was conducted in accordance with the Helsinki Declaration and was approved by the Ethics Committee of Kırıkkale University Medical School (No. 2009/046). All the subjects were informed about the study.

Blood NO levels were measured by the method of Miranda et al. which is based on reduction of nitrate, a product of NO, via the Griess reaction [7]. Lipid peroxidation was measured by total peripheral blood MDA levels [8].

TOS and TAS were measured by Erel's method [9, 10] using TOS and TAS kits (Rel Assay Diagnostics, Baran Medical, Turkey) on an Beckmann Coulter Olympus AU 400 (Beckmann Coulter, USA) biochemistry automated analyzer. The TOS results are reported in μmol H_2O_2 equivalent per liter and TAS in μmol trolox equivalent per liter. OSI was calculated by the formula

$$\text{OSI} = \text{TOS } (\mu\text{mol } H_2O_2 \text{ Eq/L}) / \text{TAS } (\mu\text{mol trolox Eq/L}).$$

Statistical analyses

Statistical evaluation of the findings were performed using Statistical Package for Social Science (SPSS) for Windows 13.0 for all statistical calculations. Chi-square, one way-analysis of variance (ANOVA), and *post hoc* Tukey HSD tests were utilized for intergroup comparisons, and Pearson's test was used for analysis of correlations between the parameters. Time-dependent changes within the patient group were evaluated by repeated measures ANOVA and the paired T-test. p-Values <0.05 were considered significant. Parametric tests were used on the grounds that all parameters had normal distribution, as demonstrated by the Kolmogorov-Smirnov test.

Results

A total of 20 first-time ischemic stroke patients were enrolled in the study as well as two control groups consisting of 27 healthy individuals and 22 pathological controls

with diabetes and hypertension. Of the 20 ischemic stroke patients, 11 (55%) were males and nine (45%) were females. Of the 27 healthy control subjects, 12 (44%) were males, 15 (56%) were females. Of the 22 pathological controls, 12 (55%) were males and 10 were (45%) females. The mean age of the patients was 64.2±14.04 years, that of the healthy control was 59.2±8.74 years, and that of the pathological control was 59.1±12.09 years. There were no statistically significant differences between the ischemic stroke and the control groups with gender and age ($p = 0.246$ and $p = 0.704$). Of the total patients, 18 individuals (90%) had a history of hypertension, whereas 18 (90%) individuals had a history of diabetes mellitus. Next, three (15%) patients were smoking and four (20%) patients were using alcohol.

The blood MDA, NO, TOS, TAS, and OSI values of the patient and control groups were monitored over 4 days (Table 1). All patients were discharged from hospital within 28 days.

In patients, there were significant changes in NO, TOS, and OSI values over time after admission. The mean hour 96 NO level was significantly lower than at hours 0, 24, 48, and 72 ($p < 0.05$, Table 2). The TOS and OSI levels of the patients were significantly higher at hour 24 than that at hours 48, 72, and 96, and the hour 48 level was significantly greater than at hour 72 ($p < 0.05$, Figure 1). There were no significant changes in MDA and TAS levels with time. Daily changes in MDA, NO, TOS, TAS, and OSI levels are summarized in Table 2.

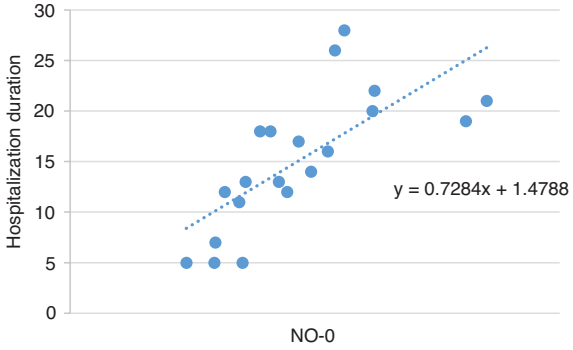

Figure 1: Correlation between NO-0 and hospitalization duration (r: 0.71; p: 0.0003).

There was also a significant positive correlation between NO at hour 0 and duration of hospitalization ($r = 0.71$, $p = 0.0003$; Figure 1).

Discussion

In this study, we found higher concentrations of MDA at all times and higher NO in patients within 0, 24, 48, 72 h in comparison with those in healthy control and pathological control groups. Conversely, TOS and OSI were lower than those in healthy control group at 96 h and those in pathological control group at 72 and 96 h. There was a positive correlation between NO at 0 h and duration of hospitalization.

Table 1: Oxidative stress metrics of patient, healthy control, and pathological control groups (mean±SD).

Tests	H. control	P. control	0 h	24 h	48 h	72 h	96 h
MDA, nmol/L	5.99±2.28	6.99±2.37	8.47±2.46[a]	7.95±1.94[a]	8.17±2.14[a]	8.39±3.13[a]	7.93±2.35[a]
NO, µmol/L	13.75±4.27[b]	8.54±3.66	18.70±6.58[a,b]	18.72±4.52[a,b]	20.21±4.69[a,b]	19.05±8.21[a,b]	12.63±3.24[b]
TOS, µmol/L	3.44±1.93	3.74±1.59	3.30±2.54	4.14±2.33	2.88±1.83	2.12±1.54[b]	2.12±1.47[a,b]
TAS, mmol/L	1.45±0.22	1.46±0.32	1.47±0.26	1.42±0.28	1.39±0.27	1.45±0.27	1.36±0.29
OSI, %	0.25±0.17	0.27±0.13	0.22±0.16	0.30±0.18	0.21±0.14	0.15±0.09[b]	0.16±0.10[a,b]

H. control, healthy control; P. control, pathological control; MDA, malondialdehyde; NO, nitric oxide; TOS, total oxidative stress; TAS, total antioxidant status; OSI, oxidative stress index. [a]$p < 0.05$ compared with healthy controls. [b]$p < 0.05$ compared with pathological controls.

Table 2: Comparison of serum oxidative stress metrics levels in patients according to days.

Tests	0 h	24 h	48 h	72 h	96 h
MDA, nmol/L	8.47±2.46	7.95±1.94	8.17±2.14	8.39±3.13[a]	7.93±2.35
NO, µmol/L	18.70±6.58	18.72±4.52	20.21±4.69	19.05±8.21	12.63±3.24[a]
TOS, µmol/L	3.30±2.54	4.14±2.33[b]	2.88±1.83[c]	2.12±1.54	2.12±1.47
TAS, mmol/L	1.47±0.26	1.42±0.28	1.39±0.27	1.45±0.27	1.36±0.29
OSI, %	0.22±0.16	0.30±0.18[b]	0.21±0.14[c]	0.15±0.09[b]	0.16±0.10[a,b]

MDA, malondialdehyde; NO, nitric oxide; TOS, total oxidative stress; TAS, total antioxidant status; OSI, oxidative stress index. [a]$p < 0.05$ compared with 0, 24, 48, and 72 h. [b]$p < 0.05$ compared with 48, 72, and 96 h. [c]$p < 0.05$ compared with 72 h.

Ischemic stroke is among the most prevalent causes of death throughout the world, and its incidence increases markedly with age. Oxidative/nitrosative stress is a well-established pathogenic mechanism in the development of neurological deficits following ischemic stroke [11].

Oxidative stress induces oxidation of lipids in blood as well as neurons, resulting in accumulation of lipid peroxides than can be measured from blood samples. This lipid peroxidation is a well-established mechanism of cellular damage and blood MDA is a reliable indicator of oxidative stress [12].

Several studies have investigated MDA levels in patients with ischemic stroke. These studies have shown that MDA levels were significantly higher in patients with ischemic stroke compared to those of controls. Ozkul et al. [13] reported higher MDA levels in patients who presented to the hospital for 48 h and suggested deleterious effects of oxidative stress on clinical outcome. In accordance with our results, however, Cojocaru et al. [14] found higher MDA levels at hour 24 and on day 7 in patients with ischemic stroke compared to a control group. Conversely, Zimmermann et al. found no significance in plasma MDA levels in patients [15]. Paspalj et al. [16] found that MDA levels were higher than those of the control group and observed that there was no time-dependent change as with our study.

While these studies [13–16] indicate substantial oxidative stress starting within days of stroke onset, the very early changes in oxidative status are less clear. Thus, in the present study, MDA levels were measured at hours 0, 24, 48, 72, and 96 post-admission. At all times, levels were significantly higher than in a healthy control group, indicating substantial lipid peroxidation due to free oxygen radical generation in the hours following stroke onset. However, there were no time-dependent changes in MDA levels over subsequent days, so MDA does not appear to be a useful index of pathogenesis or prognosis.

It is now generally accepted that NO production is enhanced at all stages of cerebral ischemia [16]. The free radical NO is an important mediator in a variety of biological processes in the brain, e.g. vascular integrity, cerebral blood flow, cerebral vasodilation, and autoregulation [17]. The role of NO at specific times after cerebral ischemia is unclear, and similar studies have yielded highly disparate results. Many previous studies have suggested that in comparison with healthy controls, NO levels were significantly greater in patients with acute stroke [13, 18–20]. Rashid et al. reported that in stroke patients the nitrate/nitrite (NO) levels were lower compared to that in the control group [21]. Samdani et al. [22] found that NO was generated by nNOSs in the acute phase of stroke but triggered by iNOS in long-term cell death, that iNOS started to increase at 12 h post-stroke reaching a maximum at hour 48, and declined to normal levels by day 7.

In the present study, the NO levels in patients with ischemic stroke were significantly higher at hours 0, 24, 48, and 72 than those in healthy and pathological control groups ($p < 0.001$). Furthermore, there was a positive correlation between initial (0 h) NO and the duration of hospital stay. Thus, early NO appears to exacerbate damage, resulting in extended hospital stay in these non-fatal cases. The fact that NO levels returned to normal levels by hour 96 indicates that protective mechanisms are ultimately activated, which begin to limit oxidative damage by hour 72. This correlation also suggests that the early NO increase is a promising target for therapy.

NO levels were lower in the high-risk control group of diabetic/hypertensive patients. Under physiological conditions, NO regulates cerebrovascular hemodynamics and has anti-inflammatory, anti-oxidative, and anticoagulant properties [23]. It is known that the release of free oxygen types directly damages the endothelial cells and vascular smooth muscles, inducing several pathological events, including inflammation [17, 23]. The causes of oxidative stress may lay the foundation for diabetes mellitus and hypertension, which include interaction of NO with types of reactive oxygen in oxidative stress, formation of lipid peroxidation products with vasoconstrictor effects, endothelial damage, increase in endothelial permeability, and stimulation of inflammation. As a result, NO levels decrease over time upon disruption of the endothelial function [24, 25]. The NO levels were significantly lower in the pathological control group in comparison with those in the healthy control group and patient group, which suggests that increased oxidative stress in hypertension and diabetes mellitus, i.e. the pathological control group, aggravates the endothelial damage and results in reduced NO levels.

The fact that NO was lower in the pathological control group suggests that chronic low levels (as well as transiently elevated levels) can contribute to the pathophysiology underlying ischemic events. Alternatively, the decrease in NO to healthy levels after 72 h and chronically low levels in the pathological group suggest possible activation of compensatory mechanisms for limiting damage due to oxidative stress.

Many previous studies have identified parameters indicative of oxidative stress that are elevated in patients with ischemic stroke. Recent studies suggest that a combined metric reflecting oxidative stress and antioxidant capacity is superior to separate indices [26]. In the present study, we first measured the serum TAS and TOS and then calculated the OSI as a measure of oxidant/antioxidant balance. While TAS did not differ among groups, TOS and

OSI levels were significantly lower in stroke patients than in the pathological control group at 72 h and lower in both control groups at 96 h, suggesting that oxidative damage starts to decrease by hour 72.

TAS reflects the capacities of all endogenous antioxidants. Gariballa et al. [27] and Cherubini et al. [28] found decreased antioxidant concentrations in ischemic stroke. Ullegaddi et al. [29] found that antioxidant capacity was restored and oxidative damage diminished in ischemic stroke patients who were administered vitamins E and C. Nanetti et al. [26] found increased total antioxidant capacity 1 month after ischemic stroke. We did not find any change in TAS levels in intergroup terms and by hours during the present study. It is possible that compensatory mechanisms responsible for reduced TOS and OSI at these times are not reflected by TAS measurement from peripheral blood.

A key consideration is how peripheral blood measures of oxidative stress reflect the equivalent processes in ischemic tissue. For instance, the blood-brain barrier may limit the spread of molecules from ischemic tissue into the blood. Small oxidant molecules like NO will freely penetrate the blood-brain barrier, while the penetration of larger molecules will depend on the degree of barrier damage. MDA and NO levels increased in the blood as anticipated; however, there were no notable changes in the levels of the other parameters. An interesting finding was that the TOS levels at hours 72 and 96 were lower in patients than in control groups. TOS values generally increase during the first 2 days post-stroke then gradually decrease over the subsequent 2 days. Considering these changes together with our MDA and NO findings, it appears that there was a notable increase in oxidative stress immediately after stroke occurrence, but this declined within 2–4 days, possibly due to tissue recovery and local/systemic defense mechanisms (although these were not reflected by increased TAS).

Limitations of this study include measurements of serum rather than tissue TAS and TOS levels, so additional studies are required to assess the relationships between these metrics and the degree of neural tissue damage, blood-brain barrier disruption, and long-term clinical outcome.

Conclusions

Additionally, further studies are needed to examine whether individual antioxidant system activities (as opposed to TAS) change post-stroke, whether free radical generation is solely responsible for the induced tissue damage, and if downregulation of antioxidant defenses are also involved. The association between the initial NO level (0 h) and duration of hospitalization not only implicates early NO accumulation in stroke severity but also identifies early NO elevation as a target for therapeutic intervention and a valuable biomarker for prognosis.

Author contributions: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.
Research funding: None declared.
Employment or leadership: None declared.
Honorarium: None declared.
Competing interests: The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

References

1. Han MH. Adams and Victor's principles of neurology. New York, USA: Mc Graw Hill Book Company, 2009:C34.
2. Rodrigo R, Fernández-Gajardo R, Gutiérrez R, Matamala JM, Carrasco R, Miranda-Merchak A, et al. Oxidative stress and pathophysiology of ischemic stroke: novel therapeutic opportunities. CNS Neurol Disord Drug Targets 2013;12:698–714.
3. Warner DS, Sheng H, Batinić-Haberle I. Oxidants, antioxidants and the ischemic brain. J Exp Biol 2004;207:3221–31.
4. Calabrese V, Bates TE, Stella AM. NO synthase and NO-dependent signal pathways in brain aging and neurodegenerative disorders: the role of oxidant/antioxidant balance. Neurochem Res 2000;25:1315–41.
5. O'Mahony D, Kendall MJ. Nitric oxide in acute ischaemic stroke: a target for neuroprotection. J Neurol Neurosurg Psychiatry 1999;67:1–3.
6. Ferretti G, Bacchetti T, Masciangelo S, Nanetti L, Mazzanti L, Silvestirini M, et al. Lipid peroxidation in stroke patients. Clin Chem Lab Med 2008;46:113–7.
7. Miranda KM, Espey MG, Wink DA. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide 2001;5:62–71.
8. Yagi K. Lipid peroxides in hepatic, gastrointestinal, and pancreatic diseases. In: Armstrong D, editor. Free radicals in diagnostic medicine. USA: Springer, 1994:165–9.
9. Erel O. A new automated colorimetric method for measuring total oxidant status. Clin Biochem 2005;38:1103–11.
10. Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin Biochem 2004;37:277–85.
11. Allen CL, Bayraktutan U. Oxidative stress and its role in the pathogenesis of ischaemic stroke. Int J Stroke 2009;4:461–70.
12. Sies H. Oxidative stress: oxidants and antioxidants. Exp Physiol 1997;82:291–5.

13. Ozkul A, Akyol A, Yenisey C, Arpacı E, Kiylioglu N, Tataroglu C. Oxidative stress in acute ischemic stroke. J Clin Neurosci 2007;14:1062–6.
14. Cojocaru IM, Cojocaru M, Sapira V, Ionescu A. Evaluation of oxidative stress in patients with acute ischemic stroke. Rom J Intern Med 2013;51:97–106.
15. Zimmermann C, Winnefeld K, Streck S, Roskos M, Haberl RL. Antioxidant status in acute stroke patients and patients at stroke risk. Eur Neurol 2004;51:157–61.
16. Paspalj D, Nikic P, Savic M, Djuric D, Simanic I, Zivkovic V, et al. Redox status in acute ischemic stroke: correlation with clinical outcome. Mol Cell Biochem 2015;406:75–81.
17. Chen Z, Mou R, Feng D, Wang Z, Chen G. The role of nitric oxide in stroke. Med Gas Res 2017;7:194–203.
18. Guldiken B, Demir M, Guldiken S, Turgut N, Turgut B, Tugrul A. Oxidative stress and total antioxidant capacity in diabetic and nondiabetic acute ischemic stroke patients. Clin Appl Thromb Hemost 2009;15:695–700.
19. Aygul R, Kotan D, Demirbas F, Ulvi H, Deniz O. Plasma oxidants and antioxidants in acute ischaemic stroke. J Int Med Res 2006;34:413–8.
20. El kossi MM, Zakhary MM. Oxidative stress in the context of acute cerebrovascular stroke. Stroke 2000;31:1889–92.
21. Rashid PA, Whitehurst A, Lawson N, Bath PM. Plasma nitric oxide (nitrate/nitrite) levels in acute stroke and their relationship with severity and outcome. J Stroke Cerebrovasc Dis 2003;12:82–7.
22. Samdani AF, Dawson TM, Dawson VL. Nitric oxide synthase in models of focal ischemia. Stroke 1997;28:1283–8.
23. Nash KM, Schiefer IT, Shah ZA. Development of a reactive oxygen species-sensitive nitric oxide synthase inhibitor for the treatment of ischemic stroke. Free Radic Biol Med 2018;115:395–404.
24. Li Q, Yon JY, Cai H. Mechanisms and consequences of eNOS dysfunctin in hypertension. J Hypertens 2015;33:1128–36.
25. Creager MA, Luscher TF, Cosentino F, Beckmann JA. Diabetes and vascular disease: pathophysiology, clinical consequnces, and medical theraphy: part I. Circulation 2003;108:1527–32.
26. Nanetti L, Taffi R, Vignini A, Moroni C, Raffaelli F, Bacchetti T, et al. Reactive oxygen species plasmatic levels in ischemic stroke. Mol Cell Biochem 2007;303:19–25.
27. Gariballa SE, Hutchin TP, Sinclair AJ. Antioxidant capacity after acute ischaemic stroke. QJM: An International Journal of Medicine 2002;95:685–90.
28. Cherubini A, Polidori MC, Bregnocchi M, Pezzuto S, Cecchetti R, Ingegni T, et al. Antioxidant profile and early outcome in stroke patients. Stroke 2000;31:2295–300.
29. Ullegaddi R, Powers HJ, Gariballa SE. Antioxidant supplementation with or without B-group vitamins after acute ischemic stroke: a randomized controlled trial. J Parenter Enteral Nutr 2006;30:108–14.