The Evaluation and Comparison of Oxidative Stress in Hemorrhagic and Ischemic Stroke

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A B S T R A C T

Background: Among different mechanisms, oxidative stress has a possible role in neural injury in cerebrovascular events.

Objectives: Assessment the oxidants-antioxidants imbalance in ischemic and hemorrhagic strokes.

Materials and Methods: Serum level of malondialdehyde, the main marker of lipid peroxidation, and total antioxidant capacity were measured in a group of 48 stroke patients consisting of 24 ischemic and 24 hemorrhagic cases with confirmed diagnosis by brain CT scan. Lesion volume and modified National Institutes of Health Stroke Scale (NIHSS) in ischemic stroke, as well as location and volume of hematoma in hemorrhagic stroke based on the first brain CT scan were determined as study variables.

Results: These two major groups did not have different oxidative profile. Low levels of total antioxidant capacity and high levels of malondialdehyde were associated with higher lesion volume in hemorrhagic stroke patients.

Conclusions: This data suggested that oxidative stress is associated with lesion volume and therefore severity of hemorrhagic stroke.

Keywords: Oxidative Stress; Malondialdehyde; Stroke

Introduction

According to WHO report, 17 million people die annually because of vascular diseases. Among them, cerebrovascular accidents are a leading cause of mortality and long-term disability (1,2). Even though 85% of all strokes are ischemic in which an artery is blocked by a clot or a plaque (3), hemorrhagic strokes (HS) are

associated with a higher rate of mortality and are generally more severe than ischemic strokes (IS) even after adjustment for relevant risk factors (4).

Several attempts have been made to understand the molecular basis of tissue damage in stroke patients (5). Special attention has been paid to reactive oxygen spices (ROS) which are involved in physiological processes like aging as well as many pathologic states such as atherosclerosis, cancer, neurodegenerative diseases, and etc (6,7). It is evident from both animal and human studies that the oxidative damage of membrane lipids and cellular proteins increases during cerebral ischemia and reperfusion (8-11).

In the ischemic process, the circulatory arrest to brain cells can cause uncontrolled activation of calcium dependent enzymes such as phospholipase A2, cyclooxygenase and neuronal nitric oxide synthase which is followed by excessive radical production (3). Although less studied, ROS and lipid peroxidation might also have a role in brain injury following HS (12,13).

Several studies in the past decade have focused on measuring oxidative biomarkers in stroke patients during the acute phase after the events and in the subsequent recovery time (14-16). Detailed biomarkers' profiles of serum, urine and CSF of stroke patients have shown a statistically significant rise in markers of oxidative damage in comparison to the controls (17).

These studies can contribute to understanding the cellular factors involved in initiation and development of stroke. Also, the possible protective role of antioxidants against brain injury can lead to new therapeutic strategies in the future for reducing mortality and morbidity among both

ischemic and hemorrhagic stroke patients (18-20).

The present study was designed to investigate the correlation of prognostic factors in stroke patients with serum levels of oxidative stress biomarkers. Until now there have been few studies that compared the differences between two types of strokes. We aimed to measure serum malondialdehyde (MDA) (21), the main byproduct of lipid peroxidation, and total antioxidant capacity (TAC) in the first 24 hours following the stroke, and to obtain evidence for their possible relationship to prognostic factors in patients with ischemic or hemorrhagic strokes.

Materials and Methods

Subjects:

A group of 24 patients with acute ischemic stroke and an equal number of patients with hemorrhagic stroke were recruited within the first 24 hours of their attack who were hospitalized at the neurological emergency ward of Ghaem hospital, Mashhad in 2014. If a patient was excluded from the study, he or she was replaced by another patient who met the inclusion criteria to reach to the determined sample volume. The diagnosis was made by the clinical examination and brain CT scan. Our excluding criteria were a previous history of a cerebrovascular event, history of a recent infectious or inflammatory disease, cancer, autoimmune disorder, hematological disorder, renal or hepatic disease, or use of immune-suppressive or anti-inflammatory drugs in the previous two months. Venous blood samples were obtained on admission. A written informed consent was provided by each patient or their relatives, and the study was approved by the

Ethics committee of Mashhad University of Medical Sciences (Ethical code: 900398).

Method:

Recorded variables included modified NIHSS (NIH Stroke Scale) (23) and consciousness on admission in all patients, as well as hematoma volume or ischemic lesion size and location in the first CT Scan (22). All examinations were conducted by the same neurologist. Lesion volume was manually estimated from digital CT images with the conventional ABC/2 formula explained by Kothari *et al.* (23) (A: the greatest hemorrhage diameter by CT, B: The diameter 90 to A, and C: the approximate number of CT slices with hemorrhage multiplied by the slice thickness).

Blood samples were centrifuged at 4°C at 3000 rpm for 10 min; and after sera were separated, they were kept at -80°C until analysis.

TAC was measured by a chemical colorimetric method using the Antioxidant Assay Kit purchased from Cayman Chemical Company, USA (Item Number 709001). This method relies on the ability of antioxidants in the sample to inhibit the oxidation of ABTS® (2,2'-azino-di-[3-ethylbenzthiazoline sulphonate]) to ABTS® •+ by metmyoglobin (24,25).

Serum MDA was measured using a colorimetric method based on the formation of adduct between MDA and thiobarbituric acid (TBA) under high temperature (90-100°C) and acidic conditions that is detectable colorimetrically at 530-540 nm. For this purpose, TBARS Assay kit was obtained from Cayman Chemical Company, USA (Item Number 10009055) (26).

Statistics:

The Statistical Package for the Social Sciences (SPSS) 16.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis and statistical significance was defined as $p<0.05$. Comparisons were performed using the Mann–Whitney U-test or Student's t-test. Kruskal-Wallis Test was used for analyzing categorical variables. Pearson correlation analysis and Spearman's test were used for correlation analysis of variables with normal distribution and abnormal distribution, respectively.

Results

Our study included 24 ischemic and 24 hemorrhagic stroke patients. Each group consisted of 11 males (46%) and 13 females (54%). Their age varied between 41 and 91 (72.5±15.5 and 69.3±13.2 in IS and HS, respectively) and there was no significant difference of age between two groups.

Serum total antioxidant level was 1.11±0.11 mmol/L in the ischemic patients and 1.14±0.14 in hemorrhagic patients; the difference was not significant (95% CI: -0.07 - 0.52, $p=0.45$).

The serum levels of MDA were 40.46±6.42 µmol/L in ischemic patients and 52.25±5.41 µmol/L in hemorrhagic patients (95% CI: -32.9 -70.8); this difference was not significant either ($p=0.24$).

Ischemic Group:

Among IS patients, the mean NIHSS was 20. There was a non-significant correlation between NIHSS and MDA (r=0.03, $p=0.791$) and no significant correlation between NIHSS and TAC (β= -0.272, $p=0.211$) .We found no significant correlation between consciousness

and TAC and MDA levels (table 1).

**Table1. Comparisons of MDA and TAC levels and consciousness in ischemic patients (N=24).**

|	Intact consciousness (N=12)	Impaired consciousness (N=12)	*p-value*
MDA (µmol/L)	46.83	34.09	0.326
TAC (mmol/L)	1.13	1.09	0.644

Hemorrhagic Group:

Among HS patients, 11 had hemorrhage in putamen, 7 in thalamus, 1 in pons and 3 had lobar hemorrhage. The size of hemorrhage was between 3-160 cm3 with a mean of 46 cm^3.

We found a significant negative correlation between TAC and lesion volume (figure 1) and a significant positive correlation between MDA levels and lesion volume in HS patients (figure 2). Patients with a higher lesion volume had a lower level of TAC (*p*=0.048) and higher mean level of MDA (*p*=0.004) than patients with lower lesion volume. We could not find any statistically significant difference between lesion location and TAC and MDA levels (*p*>0.05).
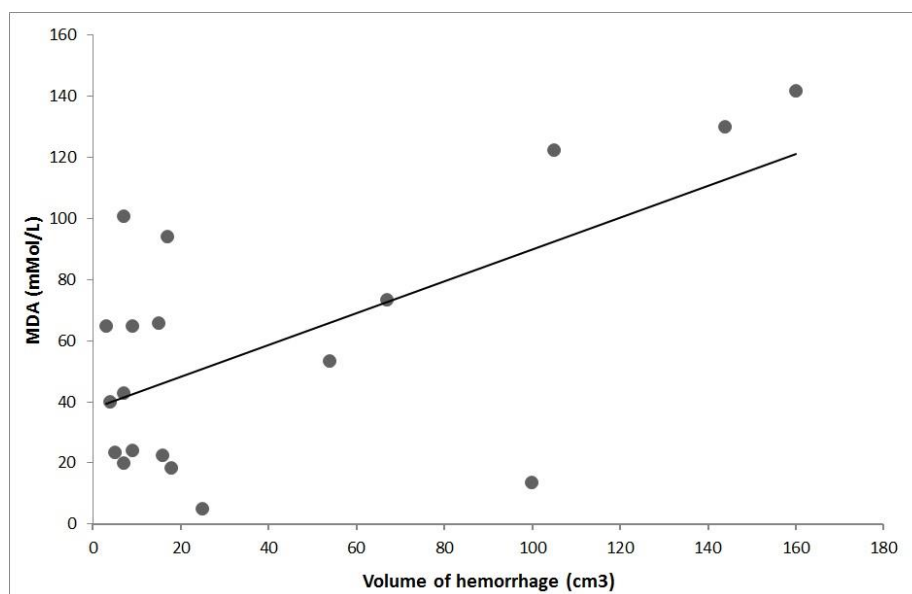
Figure1. The correlation between volumes of cerebral hemorrhage (measured by CT) and MDA level (r=0.623, *p*=0.004).
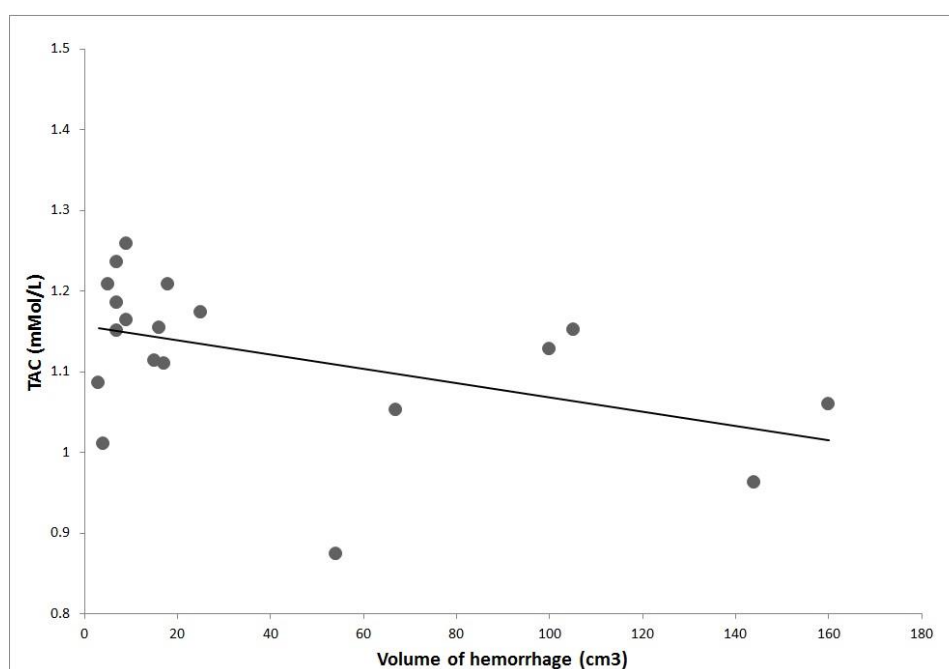
Figure 2. The correlation between volumes of cerebral hemorrhage (measured by CT) and TAC level (r=- 0.4459, *p*=0.048).

Discussion

In the present study we have focused on the relationship between serum oxidative profile and various prognostic factors of ischemic and hemorrhagic stroke. Throughout the past decades, there has been accumulating evidence of the association between oxidative stress and stroke injury, and several studies have proved oxidative damage as a mechanism underlying neuronal damage in different stages after stroke (27). Free radicals generated in the affected area of brain can exert their effect by lipid peroxidation leading to membrane changes and apoptosis, protein oxidation and impaired enzymatic functions and DNA oxidation that can eventually lead to cell death. While these factors are present in the brain in normal state and they have many important roles in signaling and as a defense mechanism against infections, they usually cannot cross the blood brain barrier and their half-life is not long enough to reach significant concentrations in serum. However following a vascular attack with or without reperfusion their modifications in plasma can reflect brain damage. Various oxidative stress markers have been previously shown to be higher in ischemic stroke patients compared to control. However, until now none of these results could suggest a different diagnostic or therapeutic approach for clinicians in practice (28-30). In an attempt to find a path between the biochemistry of stroke and its clinical management, we measured the plasma concentrations of two markers in a group of stoke patients. Malonyldealdehyde, a breakdown product of lipid peroxidation and total antioxidant capacity were chosen as indicators of the oxidant and antioxidant balance state.

This study included patients with a recent cerebrovascular accident. We assigned different prognostic factors for the two groups based on what had been previously associated with higher mortality and morbidity rate in each type. The majority of ischemic strokes are due to a thrombosis or an embolus. Previous studies have shown MDA levels are higher in ischemic patients in comparison to general population. However, few studies have focused on the correlation between stroke severity, its outcome and MDA or other markers of oxidative state. Ozkul *et al.* previously reported an association between MDA and Canadian Neurological Scale Scores (CNS). In another study, Leinonen *et al.* observed an inverse correlation of plasma total peroxyl radical–trapping potential (TRAP) and lesion volume and a significant or inverse correlation with the scores in NIHSS and HMS and a direct correlation with BI score at all-time points after the stroke in ischemic stroke (31). We could not detect any significant correlation between levels of MDA or TAC and NIHSS or consciousness. However, these levels were measured only at the initial presentation, and follow-up measurements could help to clarify MDA and TAC changes and ischemic stroke outcomes.

Stroke is a disease of different pathophysiology and hence different subtypes. It seems that hemorrhagic patients have been under represented in the literature. One reason could be its lower incidence compared to ischemic stroke. Only 15 percent of all strokes happen because of an intracranial hemorrhage (ICH). However hemorrhagic strokes are generally associated with greater severity and higher mortality after age, gender and relevant risk factors adjustments, and they are a major healthcare burden. Patients with hemorrhagic stroke are shown to have higher levels of oxidative biomarkers than control population (32). Among our 24 hemorrhagic patients 11 had lesion in putamen which is the most common location, 7 in thalamus, 1 had pontine lesion and 3 had lobar hemorrhage (1 in frontal, 2 in parietal lobe). No association between MDA levels or TAC and the location of ICH was observed. We found lesion volume to be significantly correlated with MDA levels and negatively correlated with TAC.

Comparison of MDA levels and TAC between the two major groups of stroke did not yield significant difference which is consistent with other similar studies (12). However our sample study was larger than previous studies with higher mean of NIHSS (NIHSS mean of our population in total was 21, IS=20, HS=23) and generally more severe cases.

Conclusion

This data suggested that oxidative stress is associated with lesion volume and therefore severity of hemorrhagic stroke; however, no correlation was observed between the serum level of MDA/TAC and the consciousness level as well as NIHSS in ischemic stroke. Although there is strong evidence supporting the role of oxidative stress in brain injury following a cerebrovascular attack, further research is needed to show, how it is involved in the evolution of clinical manifestations of stroke patients and its application in predicting patients' outcome.

Acknowledgment

Conflict of Interest

The authors have no conflict of interest.

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