

# Oxidative Stress and Antioxidant Markers in Serum and Red Blood Cell of Patients with Ischemic and Hemorrhagic Stroke: A Case-Control Study

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## Abstract

**Background and Aim:** Oxidative stress is a major mechanism involved in stroke pathogenesis. The present study evaluated and compared oxidative stress parameters in patients with ischemic and hemorrhagic stroke (IS and HS) at the time of hospitalization and 1 week after hospitalization.

**Methods:** This was a case-control study with 87 stroke patients (58 IS and 29 HS) as the case and 58 normal individuals as the control group. Sampling was done at the time of hospitalization and after 1 week. The serum total antioxidant capacity (TAC), thiol group, and F2-isoprostan levels were measured using Ferric Reducing Antioxidant Power (FRAP), Elman, and Enzyme Linked Immunosorbent Assay ELISA methods, respectively.

**Results:** At the time of hospitalization, there was no significant difference in TAC and F2-isoprostan levels between the case and control groups (TAC for IS =  $852.4 \pm 238.9$ , HS =  $1064.1 \pm 271.1$ , and control =  $947.6 \pm 203.1$   $\mu\text{mol/L}$ ;  $P = 0.074$ ), yet, the thiol group levels were significantly lower ( $P < 0.001$ ) in the case groups (IS and HS) compared to the control group (IS =  $360.3 \pm 139.8$ , HS =  $357.8 \pm 94.4$ , and control =  $568.4 \pm 201.4$   $\text{mmol/L}$ ;  $P < 0.001$ ). At 1 week after the time of hospitalization, the case patients showed a significant increase in thiol levels (IS at  $p = 0.005$ ; HS at  $P = 0.02$ ), yet, TAC values decreased ( $P < 0.015$ ) only in patients with HS compared to levels at the time of hospitalization. There were no significant differences between groups for antioxidant enzymes (SOD, GPx, and CAT) or the National institute of health stroke scale (NIHSS) score of patients at the time of hospitalization and 1 week later, except for superoxide dismutase (SOD), which increased in patients with HS ( $P = 0.05$ ).

**Conclusions:** The results showed that antioxidant defense status in stroke patients was lower than for the control group. Improving the antioxidant defense system, especially for HS patients by strategies such as natural antioxidant consumption is suggested.

**Keywords:** Ischemic, Hemorrhagic, Oxidative Stress, Thiol, Total Antioxidant Capacity, Antioxidant Enzyme, NIHSS

## 1. Background

Stroke or cerebrovascular accident is the third leading cause of death in the United States and the most common disabling neurological disease in adults in most places (1, 2). This disorder is caused by interruption of blood flow to a particular area of the brain. If part of the brain is deprived of adequate blood supply, brain cells will become damaged or die (2, 3). Strokes are divided pathologically to ischemic and hemorrhagic types. Ischemic stroke (IS) accounts for 87% of strokes and occurs when a blood clot or clump of fat occludes blood flow to the brain. Hemorrhagic stroke (HS) accounts for only 13% of strokes and results from rupture of a blood vessel within or around the brain (2, 4). Recent identification of several molecules in-

involved in the death of neurons have proven promising in the identification of the pathogenesis of brain damage following both ischemic and hemorrhagic strokes.

Oxidative stress is a mechanism involved in nerve damage caused by stroke (5-8) and is the result of an imbalance between the production of free radicals (reactive oxygen species) and the antioxidant defense system (1, 9). This phenomenon leads to cellular damage, cell death and acceleration of degenerative diseases associated with aging such as cancer, cardiovascular disease, diabetes, and pulmonary and neural degenerative diseases (9, 10). Increased production of free radicals and other chemical species has been confirmed both in ischemic and hemorrhagic strokes, and oxidative stress was introduced as a fundamental mecha-

nism in brain damage under these conditions (1).

Free radicals change the structure and function of target molecules by taking their electrons. Oxidants also affect cell membranes and genetic material such as DNA and RNA, and various enzymatic events, causing cell damage during ischemia and reperfusion (4, 9, 11). Despite this, a few studies have examined the effects of oxidative stress on hemorrhagic stroke (4). The current study evaluated oxidative stress parameters in the serum and red blood cell of patients experiencing ischemic and hemorrhagic strokes at the time of hospitalization and compared them with the values of these patients 1 week later.

## 2. Methods

This case-control study was conducted on an overall population of 135 individuals (58 IS and 29 HS as the case groups; 58 healthy individuals as the control group). After diagnosis and hospitalization of the patients at Valiasr hospital as well as confirmation of stroke type by a specialist, blood samples were taken both at the time of hospitalization and 1 week later. Having a background of stroke or heart attack, pulmonary, liver, kidney disease, and cancers was considered as the exclusion criteria. The control group was chosen from the healthy population, which matched for age and gender with the same exclusion criteria. Blood samples were obtained from the controls at the given time spans.

Serum samples and hemolysis from red blood cells after preparation were stored at  $-80^{\circ}\text{C}$  until testing. The serum total antioxidant capacity (TAC) was measured by ferric reducing/antioxidant power (FRAP) (12), serum F2 isoprostane level was measured by Enzyme Linked Immunosorbent Assay (ELISA) using a ZellBio kit (Germany), and thiol levels were determined using Ellman's method (13). The enzyme activity of Superoxide Dismutase (SOD) was determined using the Marklund and Marklund method (14), Catalase (CAT) using Abei's method (15) and Glutathione peroxidase (GPx) using Paglia's method (16). Hemolysate prepared from red blood cells was measured and their activity was calculated relative to the amount of hemoglobin. The NIHSS scale was used to evaluate the effect of acute stroke. The collected data was then analyzed using the SPSS 19 software with the paired t test, Analysis of Variance (ANOVA), Kruskal-Wallis, Tukey's test, Wilcoxon, one-way repeated ANOVA, and chi square test at  $\alpha = 0.05$  significance level.

## 3. Results

The average age of the patients in the IS, HS, control group was  $69.4 \pm 13.8$ ,  $67.1 \pm 16.5$ , and  $70.2 \pm 12.5$ , respec-

tively. Regarding gender there were 27 males (46.6%) in the IS group, 14 (48.6%) in the HS group, and 28 in the control group (48.3%). The remaining subjects were female. All groups were similar regarding age and gender ( $P > 0.05$ ). On the first day of the study, the mean serum TAC level showed no significant differences when comparing the IS and HS groups with the control group, although it was significantly higher in the HS group than in the IS group (1064.1 versus 854.2).

The mean thiol index was significantly lower in the IS and HS groups than in the control group. There was no significant difference in the isoprostane index between groups (Table 1). After one week, the thiol index increased in the 2 groups compared to the first day of hospitalization, yet, the serum TAC index was significantly lower in the HS group. No significant difference was observed in the isoprostane index between the first and seventh day in the 2 stroke groups (Table 2).

There were no significant differences in antioxidant enzymes activity (relative to hemoglobin) in red blood cells (RBCs) between the different groups (Table 3). The SOD level in the HS group increased from the first to seventh day (Table 4). The mean NIHSS index in the IS and HS group was  $7.11 \pm 7.63$  and  $9.93 \pm 8.92$ , respectively ( $P = 0.18$ ;  $Z = 1.33$ ). This was no significant difference between the 2 stroke groups.

## 4. Discussion

The TAC analysis showed no significant difference between the stroke and control groups, although the TAC value for the HS group was significantly greater than that of the IS group ( $P < 0.001$ ). Studies have found that TAC decreases more in post-acute stroke patients when compared to healthy controls (17, 18). The current study similarly found that TAC was lower in IS patients than in controls ( $P = 0.074$ ). In the HS group, TAC levels decreased significantly by the seventh day ( $P = 0.015$ ). Icme et al. (4) and Sheikh et al. (19) investigated oxidative stress parameters in HS and IS patients. Both reported no significant differences between stroke groups and healthy controls, although their values were lower in the case groups than in the control group.

The current findings are in contrast with those from Erel (20) concerning the HS group. They found that TAC in the HS group decreased significantly more than the control group. They used the Erel method to measure TAC and found that a defective system of free radicals may increase intracerebral hemorrhagic oxidative damage (21). The difference in TAC measurement methods could be the reason for the inconsistent results between the current study and that of Genula et al. and Ritzenthaler et al. (22), which found

**Table 1.** Comparison of the Mean Serum Antioxidant Status, Thiol and F2 Isoprostane in the Three Groups on the First Day<sup>a,b</sup>

Variable name	Group Under Study			ANOVA/Tukey's Test
	Ischemic (N = 58)	Hemorrhagic (N = 29)	Control (N = 58)	
Total Antioxidant capacity, $\mu\text{mol/L}$	852.4 $\pm$ 238.9	1064.1 $\pm$ 271.1	947.6 $\pm$ 203.1	< 0.001
Thiol groups, mmol/L	360.3 $\pm$ 139.8	357.8 $\pm$ 94.4	568.4 $\pm$ 201.4 <sup>c,d</sup>	< 0.001
F2 isoprostane, pg/mL	271/8 $\pm$ 151/3	307/7 $\pm$ 183/8	330/0 $\pm$ 153/8	0.29

<sup>a</sup>Values are expressed as mean  $\pm$  SD.<sup>b</sup> $\alpha = 0.05$ .<sup>c</sup>Ischemic.<sup>d</sup>Hemorrhagic.**Table 2.** Comparison of the Mean Serum Thiol Index and Total Antioxidant Capacity and F2isoprostane on the First and Seventh Day After Stroke in The Two Case Groups<sup>a</sup>

Group Under Study		Time		P Value Paired T-Test
		1st Day	7th Day	
Thiol, mmol/L	Ischemic (N = 58)	357.8 $\pm$ 139.9	470.1 $\pm$ 247.1	0.005 <sup>b</sup>
	Hemorrhagic (N = 29)	357.7 $\pm$ 94.4	439.2 $\pm$ 159.5	0.02 <sup>b</sup>
Total antioxidant capacity, $\mu\text{mol/L}$	Ischemic (N = 58)	852.4 $\pm$ 238.9	884.4 $\pm$ 227.4	0.410
	Hemorrhagic (N = 29)	1064.1 $\pm$ 271.1	920.1 $\pm$ 306.9	0.015 <sup>b</sup>
F2 isoprostane, pg/mL	Ischemic (N = 58)	271.8 $\pm$ 151.3	310.4 $\pm$ 171.5	0.09
	Hemorrhagic (N = 29)	307.7 $\pm$ 183.8	298.8 $\pm$ 157.3	0.66

<sup>a</sup>Values are expressed as mean  $\pm$  SD.<sup>b</sup> $\alpha = 0.05$ .**Table 3.** Comparison of the Mean Enzymes Activity of SOD/HGB, GPX/HGB and CAT/HGB in the Three Groups on the First Day<sup>a</sup>

Variable Name	Group Under Study			Anova or Kruskalwallis Test
	Ischemic (N = 58)	Hemorrhagic (N = 29)	Control (N = 58)	
SOD/HGB, IU/L	4.72 $\pm$ 5.18	4.28 $\pm$ 3.1	3.21 $\pm$ 2.69	0.36
GPX/HGB, IU/L	12.67 $\pm$ 3.5	13.61 $\pm$ 4.6	14.93 $\pm$ 5.6	0.11
CAT/HGB, (IU/L	174.6 $\pm$ 55.4	182.4 $\pm$ 63.2	181.6 $\pm$ 78.2	0.86

<sup>a</sup>Values are expressed as mean  $\pm$  SD.**Table 4.** Comparison of the Mean Enzymes Activity of SOD/HGB, GPX/HGB and CAT/HGB in Three Groups on the First and Seventh Day After Stroke in the Two Case Groups<sup>a</sup>

Group Under Study		Time		P Value Paired T-Test
		1st Day	7th Day	
SOD/HGB, IU/L	Ischemic (N = 58)	4.72 $\pm$ 5.18	4.43 $\pm$ 5.64	0.75
	Hemorrhagic (N = 29)	4.28 $\pm$ 3.1	7.37 $\pm$ 7.89	0.05 <sup>b</sup>
GPX/HGB, IU/L	Ischemic (N = 58)	12.67 $\pm$ 3.5	13.65 $\pm$ 2.77	0.11
	Hemorrhagic (N = 29)	13.61 $\pm$ 4.6	14.04 $\pm$ 4.43	0.67
CAT/HGB, IU/L	Ischemic (N = 58)	174.6 $\pm$ 55.4	181.6 $\pm$ 65.4	0.39
	Hemorrhagic (N = 29)	182.4 $\pm$ 63.2	182.6 $\pm$ 50.48	0.66

<sup>a</sup>Values are expressed as mean  $\pm$  SD.<sup>b</sup> $\alpha = 0.05$ .

that TAC decreased on the first day following IS and continued to decrease for nearly 5 days.

Studies have compared oxidative damage in HS and IS patients and healthy controls (4). Parizadeh et al. reported

that oxidative parameters are not useful predictors and evaluators of trends during the first 6 months after stroke (23). Several studies have stated that the increase in TAC after stroke may protect the victim from the harmful effects

of free radicals produced during ischemia or upon revascularization (6, 17). Nanetti et al. (7) also found that medicine can increase TAC in patients during the first month.

The thiol index decreased significantly more in HS and IS patients than in the controls ( $P < 0.001$ ). There was no significant difference between the HS and IS groups in the current study as well as in the study of Icme et al. (4). In the current study, the thiol index increased significantly in HS and IS over the first 7 days. Tsai et al. (24) reported that the amount of free thiols in acute stroke patients increased by the seventh day to a level similar to that of the control group. Several studies reported that the thiol level in IS patients was significantly lower than in the control (4, 24, 25). Thiols are organic compounds that contain a sulfhydryl (-SH) group that protects against oxidative stress.

Bektas et al. (25) proposed that thiol/disulfide hemostat is impaired in IS patients and, as the thiol level decreases any medicinal drug that contains the sulfhydryl group can increase H<sub>2</sub>S levels. Furthermore, H<sub>2</sub>S is itself is associated with vasodilatation. As a thiol and a mucolytic and neuroprotective agent, N-Acetylcysteine (NAC) is a precursor of L-cysteine and reduces glutathione. The NAC is a source of sulfhydryl groups in the cell. The antioxidant property of NAC as a sulfhydryl donor may be associated with the regeneration of endothelium-derived relaxing factor and glutathione; thus, if NAC is used to substitute thiol or alpha-lipoic acid or if the thiol-disulfide imbalance is corrected, treatment of IS can be better managed.

In the current study, there was no significant difference between groups in terms of serum F<sub>2</sub>-isoprostane levels. Beer et al. (26) maintained that oxidative stress reaches its peak on the third day after stroke. They observed a significant increase in F<sub>2</sub>-isoprostane levels in samples collected in the first phase of stroke (the first 6 hours). There was also a significant difference between the sub-groups of IS in terms of F<sub>2</sub>-isoprostane levels. F<sub>2</sub>-isoprostanes, also called 8-epi PGF<sub>2</sub> $\alpha$ , are of particular interest to researchers because they have platelet vasoconstriction activation properties as well as mitogenic properties (27).

These compounds are of non-enzymatic origin and in eicosanoid family, and are generated by oxygen radicals through random oxidation of tissue phospholipids. Several studies have indicated that isoprostanes are reliable markers for examining oxidative stress under in vivo conditions. Sanchez-Moreno et al. (27) reported that F<sub>2</sub>-isoprostane 8-epiPGF<sub>2</sub> $\alpha$  plasma was significantly greater in the stroke group than the control group. They assessed the presence of metabolites derived from prostaglandin, which were formed by random oxidation of tissue phospholipids via oxygen radicals. As markers of oxidative stress, these compounds have the potential to retract ves-

sels and swing platelet aggregations.

There is insufficient information about the type of F<sub>2</sub>-isoprostane, time interval between sample collection and centrifugation, and the storage, freezing, and de-freezing temperatures in different studies. Additionally, the isoprostane was measured in either plasma or urine in some studies. In the present study, serum F<sub>2</sub>-isoprostane was measured. F<sub>2</sub>-isoprostane disappears from the blood circulation very quickly, thus, its levels indicate continuous and discrete expression over time. Furthermore, sampling, transference, and storage of blood samples for long periods produces additional F<sub>2</sub>-isoprostane. There are several methods of measuring F<sub>2</sub>-isoprostane in body liquids, each of which measures a specific isoprostane. Moreover, different kits and methods have been used in different studies. Any of these factors can be a reason for the inconsistency between the current findings and those of other studies (28).

In this study, SOD, GPx, and CAT were investigated. No significant differences were found between groups for these parameters on the first day of hospitalization; however, after 7 days, only the erythrocyte SOD activity in HS patients had increased. The SOD is the main catalyzer of oxygen anions and dismutase them into hydrogen peroxide, thereby inhibiting the accumulation of these free radicals. Hydrogen peroxide is, in turn, trapped by CAT and GPx and converted to H<sub>2</sub>O and O<sub>2</sub> molecules. The balance between oxidative and antioxidant mechanisms of these enzymes is a critical component in cells against nerve damage associated with oxidative stress (29). Antioxidant enzymes in the plasma and red blood cells are the most important factors preventing oxidative damage caused by neurological diseases such as stroke (17).

Reports on the effects of SOD activity on acute stroke are contradictory. Similar to the current study, El Kossi et al. (2000) found no significant difference between the IS group and the control group, concerning serum SOD activity (30). On the contrary, Cherubini et al. and Demikaya et al. (1, 31) found that SOD activity decreases significantly in IS patients. Cherobini et al. (2000) reported that the levels of CAT, GPx, and SOD activity in plasma and red blood cells in patients at the onset of stroke were lower than the control group. After 1 week, CAT and GPx levels in the patient and control groups were similar. During the same time, however, SOD in the plasma was lower, and in red blood cells was higher than in the controls. The increase in SOD activity after 1 week corresponds with the results of the present study for HS patients (32). Milanlioglu et al. (29) found similar results to that of the present study; no significant differences in serum GPx was found between stroke patients and healthy controls. Demirkaya et al. (2001) found that red blood cell GPx activity decreased in the first

24 hours after the onset of stroke symptoms as compared with the control group. Aygul et al. (33) also observed that GPx activity in plasma and red blood cells decreased in IS patients.

In the current study, erythrocyte CAT activity of the red blood cells in the stroke groups showed no significant difference with the control group. In this regard, Sheikh et al. (19) reported similar findings. According to some previous reports, the different measurement methods and isoenzymes are the underlying reason for the contradictory behavior; however, further research is needed to investigate these behaviors (29, 32).

The NIHSS index was measured to evaluate the effect of acute stroke. No significant differences were found between the IS and HS groups in this regard. This finding is in line with that of Icme et al. (4). The NIHSS index is used to evaluate the effects of acute stroke on consciousness, attention, visual field, eye movement, muscle strength, speech, sensory function, and ataxias. The index is a 15-degree scale based on a neurological examination. Each patient is scored on a 0 to 5 scale where 0 indicates normal, according to his/her responses and movement abilities (34).

#### 4.1. Conclusions

This study showed that antioxidant defense status, especially the thiol index, is lower in IS and HS patients than in controls, although oxidative stress indices increased at 1 week post hospitalization. A decrease in oxidative stress and an improved antioxidant defense system, particularly from consumption of natural antioxidants, may reduce the risk of stroke and its complications.

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