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Investigation of Oxidative Stress Level and Antioxidant Enzyme Activities in Operated and Nonoperated Patients with Spontaneous Intracerebral Hematoma

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Abstract

Background Complex underlying mechanisms consisting of cytotoxic, excitotoxic, and inflammatory effects of intraparenchymal hemorrhage (ICH) are responsible for the highly detrimental effects on brain tissue. Oxidative stress also plays a significant role in brain damage after ICH; however, it is less important than other factors. In this study, we aimed to evaluate the oxidative stress parameters malondialdehyde (MDA) and antioxidant-reduced glutathione (GSH), superoxide dismutase (SOD), and catalase (CT) activities in operated and nonoperated patients with spontaneous ICH.

Methods One hundred patients with spontaneous ICH and 100 healthy controls were included in this study. Within the indication, 50 of the 100 patients underwent decompressive surgery. MDA, GSH, SOD, and CT activities were measured in the serum obtained from the patients.

Results SOD and CT levels were lower in the nonoperated group than in the operated and control groups. GSH was similar in the operated and nonoperated groups, but it was lower in the control group. However, MDA was higher in those who did not undergo surgery than in the other groups.

Conclusions In our study, MDA, an indicator of oxidative stress, was found to be lower, and CT and SOD activities were found to be higher in ICH patients who underwent decompression than in those who did not. This is the first study to present the correlations of MDA, SOD, CT, and GSH in operated and nonoperated patients with spontaneous ICH.

Keywords
- intracerebral hemorrhage
- oxidative stress
- superoxide dismutase
- catalase
- glutathione
- malondialdehyde

Introduction

Intracerebral hemorrhage (ICH) is correlated with the highest mortality and morbidity rates, although it accounts for only ~10 to 15% of all strokes.[1] Although there are many types of treatment nowadays, the disease still has fatal consequences, and there is no effective treatment option.[2] Neither the etiology nor the pathophysiology of the disease has been clearly defined yet.[3] There is a mass effect related to the hematoma and acute injury of the brain parenchyma. It is thought that one of the most important mechanisms in the pathology of ICH other than acute injury is secondary brain

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injury due to oxidative stress.[4] Increasing evidence has shown that oxidative stress levels are the main contributor to oxidative brain injury after ICH, although neuroinflammation and some antioxidant enzyme activities are involved.

The basis of oxidative stress is the formation of free radicals secondary to the metabolism in blood cells, and the most important of these free radicals are reactive oxygen species (ROS) and reactive nitrogen species (RNS).[5,6] Although these free radicals cause many neurodegenerative diseases, such as dementia and parkinsonian diseases, they also cause various focal deficits.[7,8] Oxidative stress has also been found to be associated with many psychiatric disorders.[9] Free radicals impose direct effects by causing defects in DNA or damaging the protein and lipid contents of the cell. As a result, they cause cell dysfunction or apoptosis.[5] There are three main types of ROS: superoxide radical ($\bullet O_2-$), hydroxyl radical ($^{\bullet}OH$) and hydrogen peroxide (H_2O_2), whereas RNS mainly emerge from nitric oxide (NO), nitrite dioxide (NO_2), peroxynitrite ($ONOO^-$), and their derivatives.[10,11] Malondialdehyde (MDA) is used as a marker of oxidative stress, since it has a correlation with lipid peroxidation[12].

Antioxidants are molecules that prevent oxidative stress, and they neutralize free radicals by donating one of their electrons.[13] Antioxidants can be grouped into two main groups: endogenous and exogenous. Endogenous antioxidants may be enzymatic and nonenzymatic.[14,15] Some of the enzymatic antioxidants are superoxide dismutase (SOD), catalase (CT), glutathione peroxidase (GPx), and glutathione reductase (GSH-GR).

Some studies have observed that oxidative stress products increase in both ICHs and ischemia, although there is a decrease in antioxidant enzyme activities.[16–19] The lack of explanation for the oxidative stress mechanisms in ICHs has made antioxidant therapy applications controversial, especially in terms of the prevention of secondary brain injury.

In this study, we investigated the relationship between the prognosis of ICH patients with and without operation by observing the antioxidant enzyme activities.

Material and Method

The study included 100 patients with ICH who were admitted to the emergency clinic of Van Yüzüncü Yıl University Dursun Odabaş Medical Center between January and December 2019, after consultation with the neurosurgery clinic. The ICH was proven by computerized tomography. Trauma-induced hemorrhages were excluded from the study. The study was approved by the Ethics Committee of Yüzüncü Yıl University. Fifty patients were included in the study before surgery. The study was initiated by collecting blood samples within the first 24 hours from 50 postoperative patients. Blood samples were obtained from patients with ICH who were admitted to the emergency service and considered unsuitable for decompressive surgery within the first 24 hours from the beginning of the clinic (►**Fig. 1**). As a control group, blood samples were obtained from 100 healthy people of similar ages. The blood samples were immediately centrifuged, and serum levels of SOD, GPx, GSH-GR, CT, and MDA activities were measured by a spectrophotometric method. The treatment of the patients and the scheduling of the follow-up visits was performed as per clinic standard.

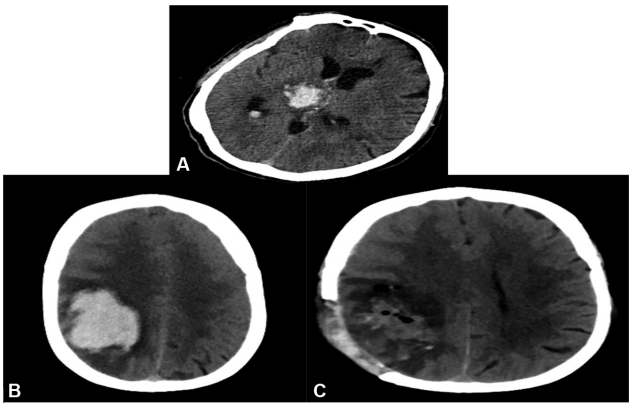


Fig. 1 (**A**) Computerized tomography (CT) of a patient with nonoperated intracerebral hemorrhage. (**B**) Preoperative CTy of a patient with intracerebral hemorrhage. (**C**) Postoperative CT of a patient with an intracerebral hemorrhage.

Determination of Superoxide Dismutase Activity

The SOD activity was measured according to the method described by Marklund et al. using spectrophotometry.[20]

Determination of Catalase Activity

The CT enzyme activity was measured according to the method developed by Aebi.[21]

Determination of Glutathione Level

All the proteins that did not carry the sulfhydryl (SH) group were precipitated with a precipitation solution. The glutathione (GSH) level was measured as the final product of the reaction.[22]

Determination of Malondialdehyde Level

The MDA level was measured according to the method developed by Gutteridge.[23]

Statistical Analysis

Descriptive statistics were expressed as mean ± standard deviation (SD), minimum, and maximum. The normality of the data distribution was tested by the Kolmogorov–Smirnov test, and the homogeneity of variances was tested by Levene's test. In terms of continuous variables in the comparisons of more than two independent groups, one-way analysis of variance (ANOVA) was used for the cases with normal distribution, and the Kruskal–Wallis test was used for the cases where normal distribution was not met. The statistical analyzes were performed with SPSS version 22.0, and $p < 0.05$ was considered statistically significant.

Results

The mean age of the patients was 63 years (range: 24–84 years); 52 patients were females and 48 were males. Within the indication, 50 patients were admitted for decompressive surgery, and 50 patients were treated conservatively. A total

Table 1 Comparative results of enzymatic antioxidants

		n	Mean ± standard deviation	Minimum	Maximum	*p* value
SOD (U/L)	Operated	50	10.7890 ± 0.68151	9.12	11.94	0.001
	Nonoperated	50	4.4582 ± 0.96402	2.37	5.77	
	Control	100	18.7279 ± 1.43692	16.25	21.04	
CT (U/L)	Operated	50	0.263000 ± 0.0211274	0.2070	0.2750	0.001
	Nonoperated	50	0.070070 ± 0.0010308	0.0680	0.0716	
	Control	100	0.247310 ± 0.0196806	0.2150	0.2870	
GSH (mmol/mL)	Operated	50	0.000034916 ± 0.0000186116	0.0000119	0.0000992	0.001
	Nonoperated	50	0.000030116 ± 0.0000183855	0.0000119	0.0000992	
	Control	100	0.000171320 ± 0.0000133741	0.0001490	0.0001980	
MDA (mmol/L)	Operated	50	0.4100 ± 0.03213	0.35	0.46	0.001
	Nonoperated	50	0.8442 ± 0.10650	0.74	1.27	
	Control	100	0.2627 ± 0.04177	0.20	0.32	

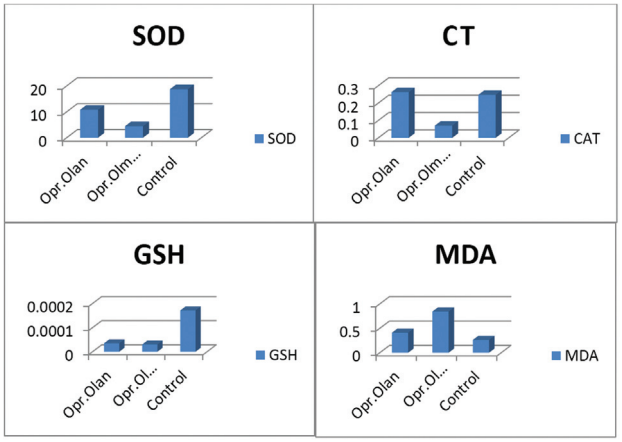
Abbreviations: CT, catalase; GSH, glutathione; MDA, malondialdehyde; SOD, superoxide dismutase.

of 12 (57%) patients who underwent decompressive surgery and 13 (45%) patients who were not operated on died.

The descriptive statistics and comparison results for SOD, CT, GSH, and MDA are shown in ►**Table 1**. The difference between the group means were found to be statistically significant. The mean values of SOD and CT were lower in the nonoperated patients than in patients operated on and in the control group. There was no difference between the mean values of GSH-GR in the operated and nonoperated groups, but the mean value of GSH-GR was lower in both groups than in the control group. However, the mean value of MDA was higher in the nonoperated group than in the operated and control groups (**Graph 1**).

Discussion

The frequency of ICH in Turkey is not exactly known. The annual incidence of ICH is 30.9/100,000[24] in the United States and 52/100,000[25] in Japan. Old age, hypertension, tobacco use, excessive alcohol consumption, previous ischemic stroke, low serum cholesterol levels, and anticoagulant use are considered risk factors for ICH.[26] Despite the developments in the treatment of hypertension and the associated decrease in the incidence of ICH,[27] the overall incidence has not changed due to the increase in hemorrhages related to increasing use of antithrombotics and cerebral amyloid angiopathy in the aging population.[27,28] ICH is still a disease with serious consequences. Brain injury in ICH is caused by direct brain damage and the mass effect of the hematoma. In the 6 months after the acute event, only 20% of the patients were able to perform daily routines independently,[29] and more than 50% died in the first year.[30] One of the most important mechanisms in the pathophysiology of ICH other than acute injury is secondary brain injury, which is believed to result from oxidative stress.[4]



Graph 1 Comparative graphics of enzymatic antioxidants (CT, catalase; GSH, glutathione; MDA, malondialdehyde; SOD, superoxide dismutase).

Reactive oxygen radicals have been found to be associated with autoimmune diseases, such as rheumatoid arthritis, neurologic diseases, diabetes mellitus, atherosclerosis, hypertension, ischemic, and cardiovascular diseases. The literature suggests that oxidative stress has an important effect on many types of cancer, including kidney, brain, lung, liver, and breast cancers.[31]

Free radicals are chemical molecules containing unshared electron pairs. At the same time, they are very reactive substances. Reactive free radicals formed in cells cause cell and tissue damage.[32] Thus, it has been determined that they play an important role in the etiopathogenesis of different diseases.[33]

In clinical and experimental studies using rats, free oxygen radical damage has been found in diseases such as bladder tumor, prostate cancer, sepsis, myocardial infarction, stroke, pregnancy, perinatal hypoxic brain injury, glomerulonephritis, uveitis, cardiopulmonary bypass, kidney transplantation, cancer, and arthritis. Reactive oxygen radicals are the source of oxidative stress. These radicals also cause DNA damage. However, antioxidants protect the cell against this DNA damage and free radicals.[34–37]

MDA is a product of lipid peroxidation and has been identified as a marker of oxidative stress since the 1960s.[12,38–40] We observed that MDA levels increased in all ICH patients. which has been reported before. Additionally, MDA levels increase in diseases such as bladder tumor, prostate cancer, and epilepsy. It has been found that the total oxidant status, which is the serum oxidative stress level, is increased in ischemic/hemorrhagic stroke.[35,41–43] In another study, it has been reported that the plasma MDA level increased in patients with Parkinson's disease.[40]

SOD is an important enzymatic antioxidants. In the study, we observed that the activities of SOD were lower in the operated and nonoperated patients than in the control group. This result is similar to those reported in other studies and is due to the deterioration of the oxidant and antioxidant balance in oxidative stress cases, such as hemorhage.[44,45] Studies that investigated the oxidant and antioxidant activities in patients with intracranial hemorrhage reported low antioxidant activities, especially in the first 24 hours after symptom onset, as in our study; this has been attributed to damage to the antioxidant system after hemorrhage.[1,46,47] However, one study found that antioxidant activities increased over time after the first 24 hours.[48] As a result, an increase in SOD may cause a decrease in oxidative stress in blood.

GSH, one of the important antioxidants is found in all mammals. It is found in very small concentrations in living organisms. At the same time, it is involved in amino acid transport and reduction of sulfhydryl groups of proteins. GSH is a nonenzymatic antioxidant. Studies have shown that the GSH levels are reduced in diseases such as bladder tumor, prostate cancer, and epilepsy.[35,41] In this study, we found that the levels of GSH, were lower in the operated and nonoperated patients compared with the control group. In this case, the brain cells showed a decrease in GSH levels due to an increased oxidative damage. Consequently, an increase in the GSH level may cause a decrease in oxidative stress in blood.

CT is an enzyme found in all living things. This enzyme converts hydrogen peroxide into water and oxygen. The highly reactive hydrogen peroxide allows it to be removed from the body. In the literature, it has been reported that CT activity is decreased in diseases such as bladder tumor, prostate cancer, and epilepsy.[35,41] Notably, our results indicated that the MDA levels were lower and the CT and SOD activities were higher in patients who underwent decompressive surgery than in patients who did not. This has not been previously reported. However, spontaneous ICH may cause oxidative stress as a result of antioxidant–oxidant imbalance. Considering that the samples were taken after surgery and 24 hours after symptom onset, this may also indicate that the oxidative balance recovered after the first day. Further, the decrease in CT activity in patients with spontaneous ICH and nonoperated patients may not be activated by H_2O_2. In such cases, decreased CT activity in the serum of patients with brain injury is observed. The conversion of H_2O_2 to H_2O and O_2 may be a cause for the decrease in CT activities. Consequently, CT accumulates in the brain cells and protects against the detrimental effects of hydrogen peroxide.

The limitations of our study include not taking into account the comorbidity of the patients, bleeding site, bleeding volume, the admission Glasgow Coma Scale score, length of stay in hospital, and drawing blood samples before surgery.

Conclusion

In this study, MDA was high and CT and SOD activities were low in the nonoperated group in patients with spontaneous ICH. However, the MDA values in the operated group were found to be lower than those in the nonoperated group. In addition, CT and SOD activities were found to be high in the operated group. The findings show that the oxidative stress level is reduced after surgery in patients with spontaneous intracranial hematoma. Thus, it can be said that secondary brain damage is reduced. Thus, patients with ICH should be taken to decompressive surgery earlier if their oxidant–antioxidant balance is severely impaired, as indicated by measurable oxidative stress markers in their serum. In addressing the limitations of this study, future studies should be conducted with more patients. The MDA, SOD, CT, and GSH levels were studied for the first time in pre- and postoperative patients with spontaneous intracranial hematoma. MDA, CT, GSH, and CT activities may play an important role in the etiopathogenesis of patients with spontaneous intracranial hematoma.

Conflict of Interest
None declared.

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