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Investigation of Total Oxidants/Antioxidants in Patients with İntracerebral Haemorrhage

İntraserebral Kanamalı Hastaların Kanında Total Oksidan/Antioksidan Durumunun Araştırılması

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Summary

Objective: Although there are numerous studies about oxidants and antioxidants in patients with ischemic stroke, the number of studies on this subject in patients with intracerebral hemorrhage (ICH) is limited. Malondialdehyde (MDA) is an oxidant parameter investigated in patients with ICH, and total oxidant status (TOS) has not been investigated so far. We aimed to investigate in blood samples the oxidant parameters MDA and TOS, and the total antioxidant status (TAS) in patients with ICH.

Material and Method: A total of 30 patients with ICH, admitted and treated at the Neurology Clinic in the Faculty of Medicine, University of Dicle and 30 control who had no stroke or any systemic disorders were included in the study. Peripheral vein blood samples taken from patients and controls were included in the first 24 hours after stroke. Serum TAS, TOS values were measured with the Erel method, a specific, fully automatic and colorimetric method, and serum level of MDA was measured with method of Ohkawa.

Results: Compared to the control group, the serum levels of TAS, TOS and MDA were significantly higher in the ICH patients ($p < 0.05$). However, there was no correlation between serum TOS, TAS and MDA levels and Glasgow Coma Scale (GCS) total scores and hematoma volumes ($p > 0.05$).

Discussion: The increase in the serum levels of MDA, TOS, and TAS in ICH patients may demonstrate that there is an increase in oxidative stress and this supports the fact that that oxidative stress may play a significant role in the pathogenesis of the ICH. However, the increase of these parameters was not found to be associated with hematoma volume and GCS in patients with ICH. *(Turkish Journal of Neurology 2013; 19:1-4)*

Key Words: Oxidative stress, intracerebral hemorrhage, Glasgow Coma Scale

Özet

Amaç: İskemik inme hastalarında oksidan ve antioksidan durumu ile ilgili çok sayıda çalışma olmasına karşın intraserebral kanama (İSK) hastalarında bu konu ile ilgili az sayıda çalışma vardır. Oksidan parametrelerden malondialdehid (MDA) İSK'lı hastalarda araştırılmış, ancak total oksidan seviye (TOS) şu ana kadar araştırılmamıştır. Amacımız İSK'lı hastaların kanında sırasıyla oksidan parametrelerden MDA ve TOS, antioksidan parametrelerinden ise total antioksidan seviye (TAS) düzeyini çalışmaktır.

Gereç ve Yöntem: Çalışmamız Dicle Üniversitesi Tıp Fakültesi Hastanesi Nöroloji Kliniği'ne başvuran ve akut İSK nedeniyle yatırılarak takip ve tedavi edilen 30 hasta ile özgeçmişinde inme ve sistemik hastalığı olmayan 30 kontrol ile yapıldı. Hasta ve kontrol grubuna ait serum TAS, TOS değerleri özgün, tam otomatik ve kolorimetrik bir ölçüm yöntemi olan Erel metoduyla ölçüldü. MDA ise serumda Ohkawa metoduna göre ölçüldü.

Bulgular: Kontrol grubuna göre İSK'lı hastalarda serum TAS, TOS ve MDA seviyeleri istatistik açısından anlamlı düzeyde yüksekti ($p<0,05$). Fakat serum TOS, TAS ve MDA düzeyi ile Glasgow koma skalası (GKS) toplam puanı ve hematom hacmi arasında korelasyon bulunmadı ($p>0,05$).

Sonuç: İntraserebral kanamalı hastaların serum MDA, TOS ve TAS seviyelerindeki artış oksidatif stresin arttığını gösterir. Bu artış İSK patogenezinde oksidatif stresin önemli bir rolü olabileceğini destekler. Ancak bu parametrelerin artışı İSK'da hematom hacmi ve GKS ile ilişkili bulunmadı. *(Türk Nöroloji Dergisi 2013; 19:1-4)*

Anahtar Kelimeler: Oksidatif stres, intraserebral kanama Glasgow Koma Skalası

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Introduction

Intracerebral haemorrhage (ICH) is a common neurologic disorder presenting with death or disability (1) and a public health problem representing 10-15% of all strokes (2). Patients with ICH have hematoma in the brain parenchyma that trigger the process resulting in neurological deficits. The mass effect of the hematoma causes edema and ischemic damage in the adjacent tissue (3, 4). Oxidation in the post-ICH hematoma is noteworthy (5) because hematoma trigger damage as a result of mechanical interruption between neurons and glia due to oligemia and mitochondrial dysfunction causing mechanical deformation (2). Free radicals are the most important factor in tissue oxidation developing after a stroke and causing brain damage (6). Oxidative processes are well known in various diseases in living organisms. The oxidation of common metabolic products, oxygen, hydrogen peroxyde, free iron and other simple compounds have been investigated (5). Blood reactive oxygen species are involved in the post-ICH oxidative brain damage. In experimental ICH models, destruction of red blood cells due to the impairment of the blood brain barrier have a toxic effect (7). Antioxidant treatment has been shown to decrease the neurologic deficit in the rat ICH model (8). Although there is an association between haemorrhage and oxidative stress, causal relationship was not clearly shown with direct evidence (9). Malondialdehyde (MDA), is used as an oxidative stress marker due to its ability to react with lipoproteins (10). Free radical mechanism may be important because of the high saturation of iron compounds forming as a result of hemoglobin breakdown in ICH. Although there is evidence of tissue damage involving free radicals following acute cerebral ischemia, the underlying pathogen mechanisms in post-ICH brain damage are not adequately understood (6, 11). Total antioxidant level (TAL) shows the overall activity of serum antioxidants (12). The degree of oxidative damage can be diminished by free radical cleansing systems. While one of the oxidant parameters, MDA, has been investigated in ICH patients, total oxidant level (TOL) has not yet been assessed. In this study we aimed to investigate the oxidative process in the pathology of ICH by assessing.

Material and Method

In this study 30 patients (11 female, 19 male) presenting at the Neurology Clinic of Dicle University Medical School Hospital between June 2010 and July 2011 were prospectively evaluated. All the patients had been diagnosed with ICH by medical history, neurological examination and neuroimaging within the first 24 hours, admitted to the hospital, treated and followed up. Patients with head trauma, a history of chronic neurologic or systemic disease were excluded from the study. All patients had routine blood chemistry performed. Patients' age, sex, hypertension, hyperlipidemia, diabetes mellitus and cardiac disease, smoking habit, antihypertensive, antidiabetic drug use were questioned. The Glasgow Coma Scale (GCS) total score was calculated for all patients. Approval was obtained from the local ethics committee. Thirty healthy age- and sex-matched (12 female, 18 male) volunteers were enrolled in the control group. History of vascular risk factors and history of concomitant medicine use was recorded at the neurology department. Patients who were using drugs containing nitrates or vasodilator drugs with nitrates as metabolic products were excluded from the study. Blood samples were collected in both the patient and the control groups, from a peripheral vein within the first 24 hours from the onset of stroke. After the blood samples were centrifuged, serum aliquots were kept at -20°C up to 24 hours, then at -50°C until the serum aliquots could be worked on. Serum TOL and TAL values were evaluated using a new automated and colorinetric measuring method developed by Erel (1,2). MDA, a product of lipid peroxidation, was measured in serum using the method of Ohkawa et al. (3).

Statistical Analysis

The results were calculated as mean ± standard values. Statistical analysis was performed with parametric methods using the SPSS 11.5 software. Differences between dual groups were investigated with independent T test and Mann-Whitney U test. The correlation of TOL, TAL and MDA with the hematoma volume and GCS in ICH patients was determined with Pearson correlation analysis. $p<0.05$ was considered to be statistically significant.

Results

The characteristics of the patients and the control group are listed in Table 1. Thirty patients (11 female, 19 male) with ICH and an average age of 64.5±15.8 were enrolled in the study; the control group included age- and sex-matched (12 female, 18 male) 30 healthy volunteer subjects with an average age of 66.3±13.9. There was no significant difference between ICH patients and the control group for age and sex ($p>0.05$). Results for TAL, TOL and MDA in ICH and control group subjects are presented in Table 2. MDA (145.30±33.0 vs. 111.30±27.4 nmol/gr, $p<0.000$), TOL (119.77±119.68 vs. 48.25±46.35 (îmol H2O2 Eq/L; $p=0.171$), and TAL (1.27± 0.40 vs 0.94±0.31 mmol Trolox Eq/L) values were respectively higher in ICH patients compared to healthy controls. Hematoma volume and total GCS score in ICH patients were 35.1± 49.7 cm3 and 10.2± 4.1, respectively. In addition, no correlation was found between serum TOL, TAL and MDA values and total GCS score and hematoma volume ($p>0.05$).

Table 1. Characteristics of patients with haemorrhage and control group

Characteristics	ICH (n =30)	Control (n=30)	P values
Age (years)	64.5 ± 15.8	66.3 ±13.9	p>0.05
Sex (F/M)	11/19	12/18	p>0.05
Obesity	12 (40%)	10 (33%)	p>0.05
Hypertension	18 (60%)	20 (67%)	p>0.05
Smoking	11 (37%)	8 (27%)	p>0.05

ICH: Intracerebral haemorrhage;
F/M: Female/Male

Table 2. MDA, TAL and TOL in patients with haemorrhage and control group

Characteristics	Patients (n=30) Mean ± SD	Control (n=30) Mean ± SD	P values
MDA (nmol/gr)	145.30±33.0	111.30±27.4	0.000
TAS (mmol Trolox Eq/L)	1.27±0.40	0.94±0.31	0.001
TOS (îmol H2O2 Eq/L)	119.77±119.68	48.25±46.35	0.006

MDA: Malondialdehyde; TAL: Total antioxidant level; TOL: Total oxidant level
SD: Standard deviation

Discussion

We determined the oxidant (MDA, TOL) and antioxidant (TAL) parameters in ICH patients, and found that these levels increased in the serum in the acute stage in ICH patients. These findings may show that oxidative stress develops following ICH.

Reactive oxygen species are well known to play a mediator role following oxidative brain damage. The significant role of oxidative stress in ICH pathogenesis is well established (4,5). Experimental studies are performed on the role of oxidative stress in ICH for the prevention of secondary damage developing post-ICH (5, 6). There are very few studies on the oxidant and antioxidant parameter levels in the serum of ICH patients. Parizadeh et al. have found that although pro-oxidant/anti-oxidant ratio increases in the serum of stroke patients, there is no difference between ischemic and hemorrhagic stroke subgroups and the oxidative parameter does not have a predictive benefit for 6 monthly prognosis (7). Alexandrova et al. suggested that lipid peroxide levels increased in the serum of ICH patients and lipid peroxide levels were associated with worse prognosis (8). We did not find a correlation between serum TOL, TAL and MDA values and GCS total score and hematoma volume ($p>0.05$); the reason may be the small sample size. Aygül et al. study similarly did not find a correlation between the hematoma value and baseline GCS total score and oxidative stress markers (9). While another study showed a positive correlation between ascorbic acid, an antioxidant and GCS total score, there was a negative correlation between hematoma volume and ascorbic acid level (10). Both experimentel and clinical studies show that the formation of free radicals causing oxidative stress play an important role in the pathogenesis of brain damage seen following haemorrhagic stroke. There is strong evidence that shows that free radical production following haemorrhagic stroke is one of the important mechanisms of brain damage (11,12). Free radicals and lipid peroxidation are involved in the pathophysiology of stroke (13,14). Brain tissue, for various reasons, is particularly sensitive to the noxious effects of free radicals (11,12). High levels of oxidant or antioxidants in serum do not necessarily mean high levels in the brain. However, mainly oxidant level increase may show oxidative stress resulting from any systemic cause. Oxidative stress developing indirectly in the brain due to impairment of the blood brain barrier in ICH may reflect on serum levels (15). Although oxidant and antioxidant levels have been in ischemic stroke patients have been investigated widely, there are few studies on this subject in haemorrhagic stroke patients (16). In our study serum MDA, TOL and TAL values in ICH patients were clearly higher than those in the control group. While MDA and TOL values are expected to increase in ICH due to tissue damage, we believe TAL values may have risen compensatorily due to oxidative stress. Chen and Zhou found that lipoperoxide levels showing oxidative stress in 351 ICH patients were significantly higher than the control group (16). Similarly, in our study we found oxidative stress markers including TOL and MDA to be higher than those in the control group. In the study Aygül et al. conducted with 17 ICH patients, there was no significant difference for MDA levels between controls, but another oxidant parameter, nitric oxide levels were significantly higher in ICH patients (9). Our study is the first one investigating TOL in the blood of ICH patients. Oxidative brain damage and antioxidant treatments were reported to diminish neurological deficit in the rat ICH model but the oxidative stress source was not exactly determined (17,18). Concurrent increase in serum MDA, TOL and TAL values in ICH patients show increase in oxidative stress. These findings support the role of oxidative stress in the pathogenesis of ICH. However, the absence of a correlation between these parameters and hematoma volume and GCS total score in ICH indicates that oxidative stress markers do not have a prognostic value.

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