LOW SERUM TOTAL ANTIOXIDANT STATUS MAY REFLECT THE SEVERITY OF NEUROLOGICAL IMPAIRMENT IN PATIENTS AFTER SPONTANEOUS INTRACEREBRAL HEMORRHAGE

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ABSTRACT

The purpose of this study was to explore the relationship between the serum total antioxidant status (TAS) and the severity of neurological deficit in patients with acute spontaneous intracerebral hemorrhage (sICH). It was found that TAS correlated with the severity of neurological impairment, evaluated by both the NIHSS and the Mathew Stroke Scale. Furthermore, the parameter had the lowest values in patients who died, and the highest values were found in the group of patients who made *a good recovery* according to the Glasgow Outcome Scale. Future studies should show whether TAS may serve as a reliable predictor of outcome after sICH.

Key words: *spontaneous intracerebral hemorrhage, oxidative stress, total antioxidant status, peripheral blood*

INTRODUCTION

Spontaneous intracerebral hemorrhage (sICH) accounts for only 15-20% of all strokes but in comparison with them it causes the highest degree of disability and lethality [7]. In contrast to ischemic stroke, the role of oxidative stress in hemorrhagic stroke-induced brain injury has been insufficiently investigated. Limited data have shown the presence of DNA and protein oxidative damage in the brain of experimental models [11]. Few data exist on increased lipid peroxidation level in erythrocytes and serum of sICH patients [13,14]. Furthermore, the level of serum lipid hydroperoxides predicts severe disability in hemorrhagic stroke survivors [2]. The published data on the antioxidant enzymes activity are contradictory [4,13]. To the best of our knowledge, there is no study published in which the serum total antioxidant status has been estimated as an indicator of the severity of neurological deficit after sICH.

The objective of the present study was to explore the possible relationship between the serum TAS level and the severity of neurological deficit in patients after acute spontaneous intracerebral hemorrhage.

MATERIAL AND METHODS

Patients

Thirty nine patients, admitted within 48 hours of stroke onset to the Department of Neurology, University Hospital - Pleven, were selected. The study did not include individuals with a hemorrhage due to a brain tumor, trauma, hemorrhagic transformation of cerebral infarction, rupture of an aneurysm or arteriovenous malformation. No individuals with intraventricular spread of blood or subarachnoidal hemorrhage, acute or chronic infections, cancer, kidney and liver diseases or past surgical procedures were included. A detailed questionnaire assessing the medical history and physical state of the patients was filled out by an experienced neurologist. Data regarding demographic and risk factors, and clinical neurological symptoms of the study population were prospectively collected.

All experiments were conducted in accordance with the rules and regulations approved by the University Research Ethics Committee.

Neurological examination

Computer tomography scan of the brain was performed on admission. The patients' neurological deficit was evaluated by means of the Mathew Stroke Scale (MSS) and the National Institutes of Health Stroke Scale (NIHSS) on admission. The Glasgow Outcome Scale (GOS) was used to evaluate the clinical outcome at discharge.

Biochemical tests

Peripheral venous blood was obtained at hospital admission. Blood biochemical analysis was performed using standard laboratory methods. The collected sera were stored at –20°C until assayed. TAS was measured by the method of Erel [9] on an automated analyzer using an assay kit (Mega Tip San ve Tic Ltd Sti). In short, antioxidants in the sample reduce dark blue-green ABTS radical to colorless reduced ABTS form. The change of absorbance at λ=660 nm is related with total antioxidant level of the sample. The assay is calibrated with a stable antioxidant standard solution which is traditionally named as Trolox Equivalent that is a vitamin E analog. The results are expressed in mmol Trolox Equivalent /l.

Statistical analysis

The statistical analysis was performed with the Statistical Package for Social Sciences 14.0 (SPSS, Chicago, IL, USA). Normality of distribution was checked with the Shapiro–Wilk test. The interval variables were represented as mean (± standard deviation, SD) or median (25th–75th percentile) depending on the type of distribution. The significance of differences between groups was assessed by one-way ANOVA for normally distributed data along with the Kruskal–Wallis test for non-parametric data. Spearman or Pearson's product moment correlations were used to examine the relationships between clinical parameters and TAS. A value of $p < 0.05$ was considered statistically significant.

RESULTS

The baseline characteristics of the patients included in the present study are given in Table 1.

Table 1. Baseline characteristics of sICH patients

Parameter	**Patients n=39**	
Age [years], (SD)	65	(12)
Male sex, n (%)	14	(36)
Vascular risk factors		
Arterial hypertension, n (%)	38	(97)
Diabetes mellitus, n (%)	3	(8)
Alcohol abuse, n (%)	27	(69)
Smoking, n (%)	12	(31)
Neurological examination		
MSS on admission, median (25th–75th percentile)	36	(14-51)
NIHSS on admission, median (25th–75th percentile)	22	(18-28)
GOS on admission, median (25th–75th percentile)	3	(1-3)
Biochemical tests on admission		
WBC [$x10^9$/l], (SD)	10.6	(3.8)
Glucose [mmol/l], median (25th–75th percentile)	6.8	(5.9-7.6)
Total cholesterol [mmol/l], (SD)	5.57	(1.08)
Fibrinogen [g/l], (SD)	3.18	(0.89)
Triglycerides [mmol/l], median (25th–75th percentile)	1.12	(0.76-1.47)

n – number of patients, SD – standard deviation; GOS – Glasgow Outcome Scale, NIHSS – the National Institutes of Health Stroke Scale, MSS- Mathew Stroke Scale, WBC – white blood cells.

Fourteen (36%) of the 39 patients were males. The mean age of the participants was 65±12 years. The neurological deficit on admission (median, 25th–75th percentile) assessed by MSS was 36 (14-51) and by NIHSS was 22 (18-28) points. The clinical outcome according to GOS was 3 (1-3). Thirty-one percent of the patients died within the first week of stroke onset, 56 % had moderate to severe disability, and only 13 % made a good neurological recovery.

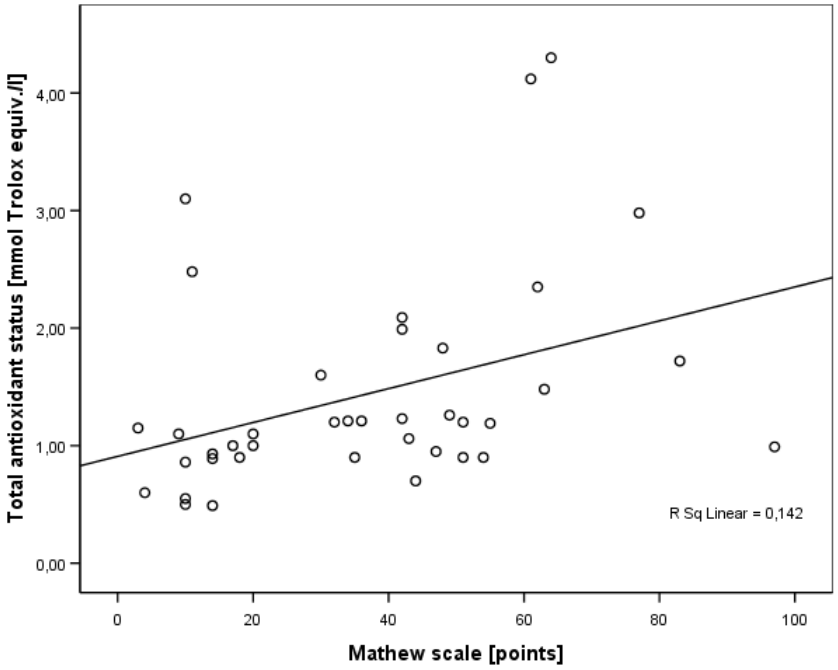

Fig. 1. Correlation relationship between the total antioxidant status and the severity of neurological impairment assessed by the Mathew Stroke Scale.

It was found that TAS correlated with the neurological deficit severity assessed by both the Mathew Scale (R_s=0.441, p=0.005) (Fig. 1) and NIHSS (R_s=-0.368, p=0.021) (Fig. 2). The serum TAS decreased with the increase in the deficit severity. Furthermore, the parameter had the lowest values in patients who died, and the highest values were found in the group of patients who made a good recovery according to the Glasgow Outcome Scale (Kruskal-Wallis, $\chi 2$=6.029, p=0.049) (Fig. 3).

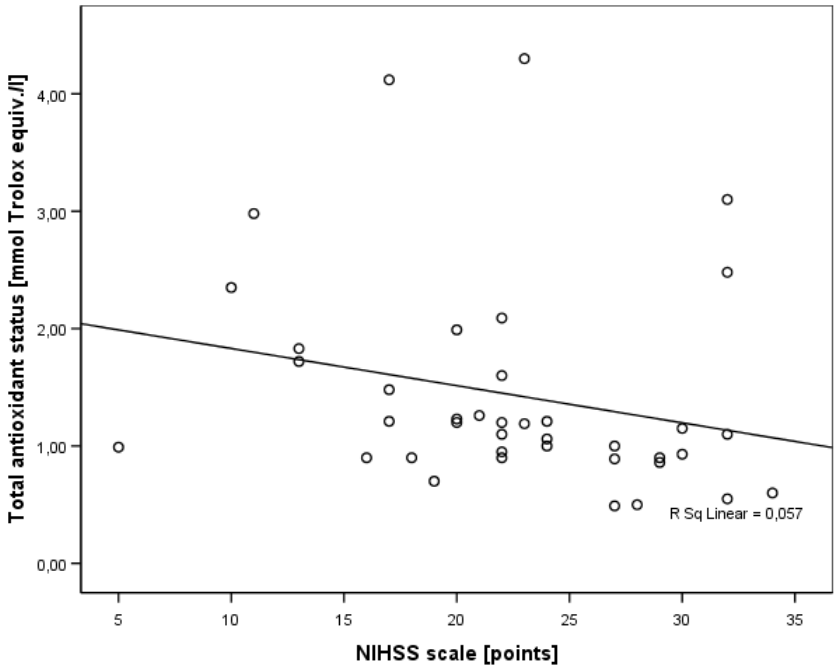

Fig. 2. Correlation relationship between the total antioxidant status and the severity of neurological impairment assessed by NIHSS.

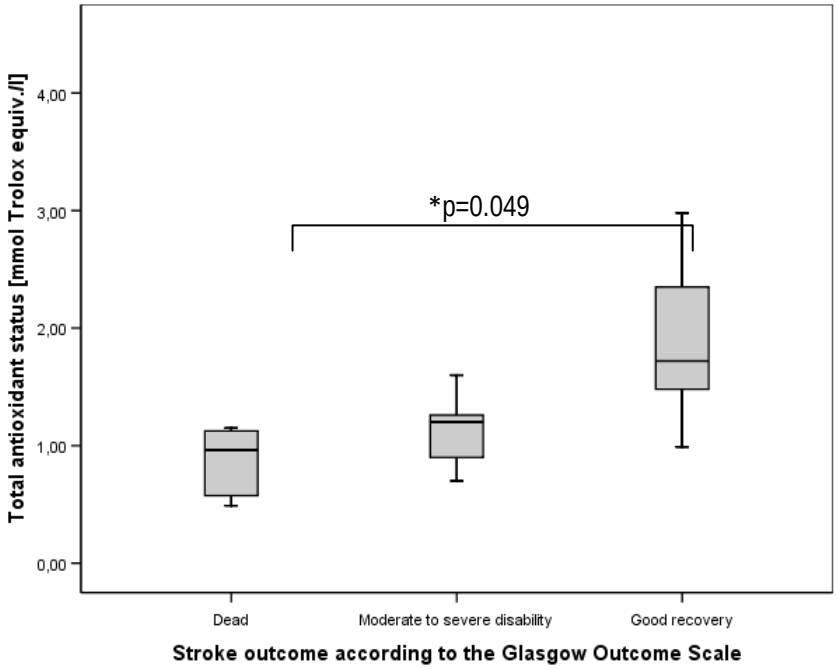

Fig. 3. Serum total antioxidant status in relation to clinical outcome

DISCUSSION

Reactive oxygen species (ROS) production is an integral part of human metabolism. As ROS have the potential to cause oxidative damage by reacting with biomolecules, they are implicated in the process of ageing and pathophysiological mechanisms of various disease states. Living organisms have developed a complex antioxidant system to protect against the deleterious effects of free radicals. Under certain circumstances, when the prooxidant/antioxidant balance is shifted in

favor of the prooxidants, the harmful condition known as oxidative stress occurs. The variety of antioxidants in the serum makes it difficult to measure each antioxidant separately. Furthermore, evidence suggests that a state of synergism and cooperation exists between different antioxidants. That is why, it is desirable to evaluate the serum total antioxidant status as a complex parameter reflecting homeostasis of redox metabolism along with the key antioxidants.

Published data on the role of oxidative stress in the pathophysiological mechanisms of injury after sICH are scarce [2,3]. A few studies have reported that blood antioxidant defense capacity changes after hemorrhagis stoke [1,13]. The plasma levels of ascorbic acid and retinol are reduced after sICH [14] and the reduction is inversely related to hematoma volume and degree of clinical deterioration. Total superoxide-scavenging activity of plasma is decreased in acute hemorrhagic stroke, as well [4]. High levels of uric acid (UA), a metabolic by-product with significant antioxidant activity, have been reported to correlate with the severity of neurological deficit and poor outcome [10]. Some data on the role of TAS in different clinical states have been published [5,6]. To the best of our knowledge, there are no results reported on the role of serum TAS in predicting clinical severity and outcome of sICH.

In the present work, we found that the serum TAS level reflected both the severity of neurological deficit and the disease outcome. What is more, the low TAS proved to be an indicator of poor prognosis and lethality thus supporting the idea of ROS participation in the pathophysiological mechanisms of stroke. The reduced antioxidant defense capacity may cause immune dysfunction, which in turn may contribute to the poor clinical outcome [8, 15].

Serum TAS is mainly related to low-molecular antioxidants, in particular SH-groups of proteins (albumin), UA, ascorbic acid, alpha-tocopherol and billirubin. It should be mentioned here that the production or consumption of any antioxidant may influence the total antioxidant capacity. Therefore, careful interpretation of the results is necessary. Thus, for instance, UA concentration in serum is comparatively high and is considered to significantly contribute to the TAS levels measured. At the same time, the levels of UA increase in a variety of disease states accompanied by oxidative stress. Under conditions of local ischemia, for example, an increased UA production and increased radical generation have been detected. Obviously in such cases pro-oxidant and pro-inflammatory effects of UA overwhelm its antioxidant properties and the increase in TAS will reflect the presence of oxidative stress. Serem UA levels have been independently related to an increased risk of death after sICH [10]. The results obtained in the present work showed, however, that the serum UA levels of patients were not responsible for the TAS levels registered since they decreased with the severity of neurological impairment. Hence, some other antioxidants, vitamin E and C particularly, may cause a reduction in the observed antioxidant status levels. This is further supported by findings such as reduced plasma concentration of ascorbic acid, α-tocopherol and protein thiols correlating with the degree of neurological deficit after sICH [4].

A possible limitation on the use of TAS as an oxidative stress biomarker in clinical practice is that the antioxidant status is measured in serum and may not adequately reflect the level of the intracellular antioxidants in the target area – the injured brain tissue by sICH.

CONCLUSION

The data obtained show that: 1. TAS rapidly decreases in response to hemorrhage-initiated ROS overproduction; 2. Serum antioxidant activity is an important factor facilitating protection against stoke-originated oxidative stress. Future experimental and clinical studies should show whether TAS may serve as a reliable predictor of lethality and clinical outcome after sICH.

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