Association between oxidative stress biomarkers and concentrations of some metal ions in the blood of patients with brain tumors and hydrocephalus

Ljiljana Vujotić[1,2], Siniša Matić[2], Slavica Borković-Mitić[3], Aleksandar Stojsavljević[4], Jelena Mutić[4], Vladimir Baščarević[1,2], Miloš Joković[1,2], Slađan Pavlović[3]

[1]Department of Surgery, Faculty of Medicine, University of Belgrade, Belgrade, Serbia
[2]Clinical Center of Serbia, Neurosurgery Division, Belgrade, Serbia
[3]Department of Physiology, Institute for Biological Research "Siniša Stanković",
 University of Belgrade, Belgrade, Serbia
[4]Faculty of Chemistry, University of Belgrade, Belgrade, Serbia

Corresponding author:
Dr Slavica Borković-Mitić
Department
of Physiology
Institute for
Biological Research
"Siniša Stanković"
University
of Belgrade
Bulevar despota
Stefana 142
11060 Belgrade, Serbia
Phone: +381 11 2078 341
E-mail: borkos@ibiss.bg.ac.rs

Abstract

Introduction: Any substance that induces production of free radicals can be a potential cause of brain damage. The aim of our study was to investigate the relationship between some metal ions and oxidative stress biomarkers in the blood of patients with brain tumor and hydrocephalus.
Material and methods: Our study included 27 control subjects, 24 patients with brain tumor and 21 patients with hydrocephalus. The activities of superoxide dismutase (CuZn SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione reductase (GR), glutathione S-transferase (GST) and acetyl cholinesterase (AChE), as well as concentrations of reduced glutathione (GSH), lipid peroxides (TBARS) and sulfhydryl groups (SH) were analyzed in the plasma and red blood cells (RBCs) of patients. We also determined the concentrations of Mn, Ni, Co, Cu, Zn, As, Se, Cd, Hg and Fe.
Results: The higher activity of SOD and concentration of GSH in both investigated groups could indicate higher oxidative stress. We also observed decreased levels of SH groups in both groups of patients. In both groups of patients we detected decreased concentrations of Ni, Co, Zn and Fe (and Mn in brain tumor patients) and increased concentrations of As, Se and Cd in the blood. Interestingly, we observed a higher concentration of Cd in both plasma and RBCs of hydrocephalus patients compared to the patients with brain tumor.
Conclusions: There are strong correlations between some metal ion concentrations and certain oxidative stress biomarkers in the blood of patients, which supports our hypothesis, but the observed trend needs to be further investigated.

Key words: antioxidant enzymes, trace elements, neurotoxicity redox homeostasis.

Introduction

Oxygen is an inevitable component of aerobic life. Incomplete reduction of oxygen to water during normal aerobic metabolism generates reactive oxygen species (ROS), which have one or more unpaired electrons. ROS, such as superoxide anion, singlet oxygen, hydroxyl radical

AMS

and hydrogen peroxide, play an important role in many pathophysiological processes. ROS are also involved in oncogenes, oxidative stress and cellular differentiation [1]. Oxidative stress is defined as an imbalance between the production and removal of ROS. The cellular antioxidant defense protects cells against ROS and includes enzymes, such as superoxide dismutases (SODs), catalase (CAT), glutathione peroxidases (GSH-Px), glutathione reductase (GR) and glutathione S-transferase (GST), as well as non-enzymatic antioxidants, such as glutathione (GSH) [1].

Many studies have reported toxic and carcinogenic effects induced by certain metal ions. It is also known that some essential transition metal ions (zinc, iron, copper, cobalt and manganese) participate in the control of various metabolic and signaling pathways. A complex balance of trace elements is crucial for all areas of maintaining human health, preventing health problems and overcoming them [2]. Trace elements are important for development of the nervous system, myelination of nerve fibers and neuronal excitability [3]. For normal trace element homeostasis in the brain the blood-brain barrier plays an important role [4].

The brain represents 2% of the total body, but utilizes 20% of oxygen consumed by the body, indicating that the brain can be a source of many more free radicals than the other body tissues [5]. Oxidative damage has long been implicated in the process of carcinogenesis, as well as in the degree of malignant transformation of most types of tumors [4]. Hydrocephalus represents an abnormal accumulation of cerebrospinal fluid (CSF) within or around the brain, due to obstruction of CSF flow, inadequate CSF absorption and/or excessive CSF production. Untreated hydrocephalus can lead to ventricular dilatation and increased intracranial pressure, potentially causing an enlarged cranium (in children), brain atrophy, neuronal deficits and eventually death [6]. Abnormal metabolism of metal ions (known to participate in oxidative cascades) is associated with hydrocephalus in farm animals [7].

There are only a few reports on the involvement of ROS in the development of brain tumors and hydrocephalus in correlations with some metal ion concentrations. Thus, the aim of the present study was to investigate the possible relationship between oxidative stress biomarkers and some metal ions in the blood of patients with brain tumors and hydrocephalus. We investigated the activity of the following antioxidant defense enzymes: copper and zinc-containing superoxide-dismutase (CuZn SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), glutathione peroxidase (GSH-Px, EC 1.11.1.9) and glutathione reductase (GR, EC 1.6.4.2) in red blood cells (RBCs), and activity of glutathione S-transferase (GST, EC 2.5.1.18) in the plasma. We also determined plasma total glutathione content (GSH) and concentration of sulfhydryl groups (SH). As an indicator of lipid modification, we measured plasma lipid peroxide concentration (TBARS) and acetylcholine esterase activity (AChE, EC 3.1.1.7) as biomarkers of neurotoxicity.

Material and methods

Subjects

In our study, we used blood samples obtained from clinically healthy persons (control group), patients with various forms of malignant brain tumors and patients suffering from hydrocephalus. All brain damage was confirmed clinically, histologically or using magnetic resonance.

Blood samples of 27 healthy control subjects (mean age: 47 ±3 years; sex ratio m/f = 14/13) were used in our experiments. Blood samples were also obtained from 45 patients with intracranial tumors (mean age: 41 ±6 years; sex ratio m/f = 15/9) and hydrocephalus (mean age: 43 ±5; sex ratio m/f = 13/8). Several types of malignant tumors were included in our study: 17 glioblastoma multiforme WHO grade IV (GBM), two medulloblastoma WHO grade IV, two atypical teratoid rhabdoid tumor (ATRTs), one meningioma atypicum WHO grade II and two secondary-metastatic tumors (melanoma, carcinoma). All the tumors were classified according to the criteria of the WHO [8].

The exclusion criteria in the study were viral infections, dietary supplements and smoking. The study was conducted in full compliance with principles of the World Medical Association Declaration of Helsinki "Ethical Principles for Medical Research Involving Human Subjects" (Helsinki, 1964, as amended during 1975–2000); and current legislative and government regulations of the Republic of Serbia. The study has been approved by the Ethics Committee of the Clinical Center of Serbia Belgrade. Blood samples were taken from all subjects only after obtaining written informed consent for participation in the study. All data and the personal information collected in this study are subject to medical confidentiality and may only be brought together for processing and evaluation in an anonymous form.

Blood sampling and biochemical procedures

Each participant donated a 5 ml blood sample. Lithium-heparin was used as an anticoagulant. Immediately after collection, blood samples were centrifuged at 5000 rpm for 15 min in order to separate plasma and RBCs.

Oxidative stress biomarkers were assessed immediately after blood collection in order to avoid any possible modification of the results caused by

storage [9]. Activities of CuZn SOD, CAT, GSH-Px and GR were determined in the RBCs, while activities of GST and AChE, as well as concentrations of total GSH, SH groups and lipid peroxides (TBARS) were determined in the plasma.

GST activity in the plasma towards 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate was measured as described by Habig *et al.* [10] and expressed as nmol GSH/min/ml of plasma. GSH concentration was determined in the plasma according to the method of Griffith [11] and expressed as nmol/l plasma. The concentration of SH groups was determined in the plasma according to the method of Ellman [12] and expressed as µmol/ ml of plasma. AChE activity was determined spectrophotometrically at 412 nm in the plasma according to the Ellman *et al.* [13] method. AChE activity was expressed as µmol/min/l plasma. Plasma lipid peroxidation was measured by the thiobarbituric acid reaction according to the method of Ohkawa *et al.* [14] at 532 nm and expressed as nmol TBARS/ml of blood.

Isolated RBCs were washed three times with 2 volumes of isotonic 0.9% NaCl. The hemoglobin (Hb) concentration in RBCs (g Hb/100 ml) was estimated by the cyanmethemoglobin method [15] and used for calculating enzyme activities. CuZn SOD activity was measured in the RBCs from which Hb was previously removed by the method of Tsuchihashi [16]. CuZn SOD activity was measured by the epinephrine method [17] and one unit of SOD activity was defined as the amount of enzyme that caused 50% inhibition of the autoxidation of adrenaline to adrenochrome. CAT activity was determined according to Beutler [18] and expressed as µmol H_2O_2/min/g Hb. In accordance with [19], hemolysates containing about 50 g Hb/l were used for the determination of GSH-Px activity [20]. GSH-Px activity was expressed as nmol NADPH/min/g Hb. The activity of GR was estimated by measuring NADPH oxidation at 340 nm [21] and expressed as nmol NADPH/min/g Hb.

The oxidative stress biomarkers were measured simultaneously in triplicate for each sample, using a Shimadzu UV-1800 spectrophotometer with a temperature-controlled cuvette holder. All chemicals were purchased from Sigma-Aldrich (Saint Louis, MO, USA) or Merck (Darmstadt, Germany).

Metal analysis

The concentrations of nine elements (Mn, Ni, Co, Cu, Zn, As, Se, Cd, and Hg) were determined by inductively coupled plasma-mass spectrometry, ICP-MS (ICAP Q, Thermo Scientific X series 2). The entire system was controlled with the Qtegra Instrument Control Software.

The iron was determined by ICP-optical emission spectroscopy, ICP-OES (model 6500 Duo,

Thermo Scientific, UK). The entire system was controlled with the iTEVA software.

Sample preparation

Plasma and RBCs were transferred into PTFE cuvettes and measured. Four milliliters of 65% nitric acid and 1 ml of 30% hydrogen peroxide were added to each PTFE cuvette and digestion was performed under the following program: warm up for 2 min to 85°C, 4 min to 135°C, 5 min to 230°C and held for 15 min at that temperature. After cooling samples were quantitatively transferred into volumetric flask (10 ml) and diluted with distilled water.

Statistical analysis

The data were expressed as mean ± SE. The degree for statistical significance was defined as $p < 0.05$. Data were checked for normality using the Kolmogorov-Smirnov test. Differences in investigated parameters between groups were calculated using one-way ANOVA. Post hoc pair-wise comparisons were performed using the Tukey HSD test. Principal component analysis (PCA) was implemented to statistically determine the differences between investigated groups based on all investigated oxidative stress biomarkers. Data considering metal concentrations were analyzed by the Mann-Whitney *U* test and expressed as mean ± SD. Relationships among the analyzed parameters were investigated using Pearson's correlation coefficients. The statistical package Statistica 10.0 was used for all analyses.

Results

The activity of CuZn SOD in RBCs (Figure 1) was significantly higher in both brain tumor and hydrocephalus groups compared to the control individuals ($p < 0.05$).

However, the activities of CAT, GSH-Px and GST (Figure 1) were not significantly different in the investigated groups when compared to the controls. On the other hand, GSH concentration (Figure 2) was significantly higher in both the brain tumor and the hydrocephalus groups ($p < 0.05$). Significantly higher AChE activity (Figure 2) was observed in hydrocephalus patients when compared to both the controls and the brain tumor group ($p < 0.05$). Significantly lower concentration of SH groups (Figure 2) was detected in both the brain tumor and the hydrocephalus groups ($p < 0.05$) when compared to the control individuals.

Principal component analysis (PCA) was employed to detect a possible separation of all three examined groups of individuals based on overall investigated oxidative stress biomarkers. The summary results of PCA for all three investigated
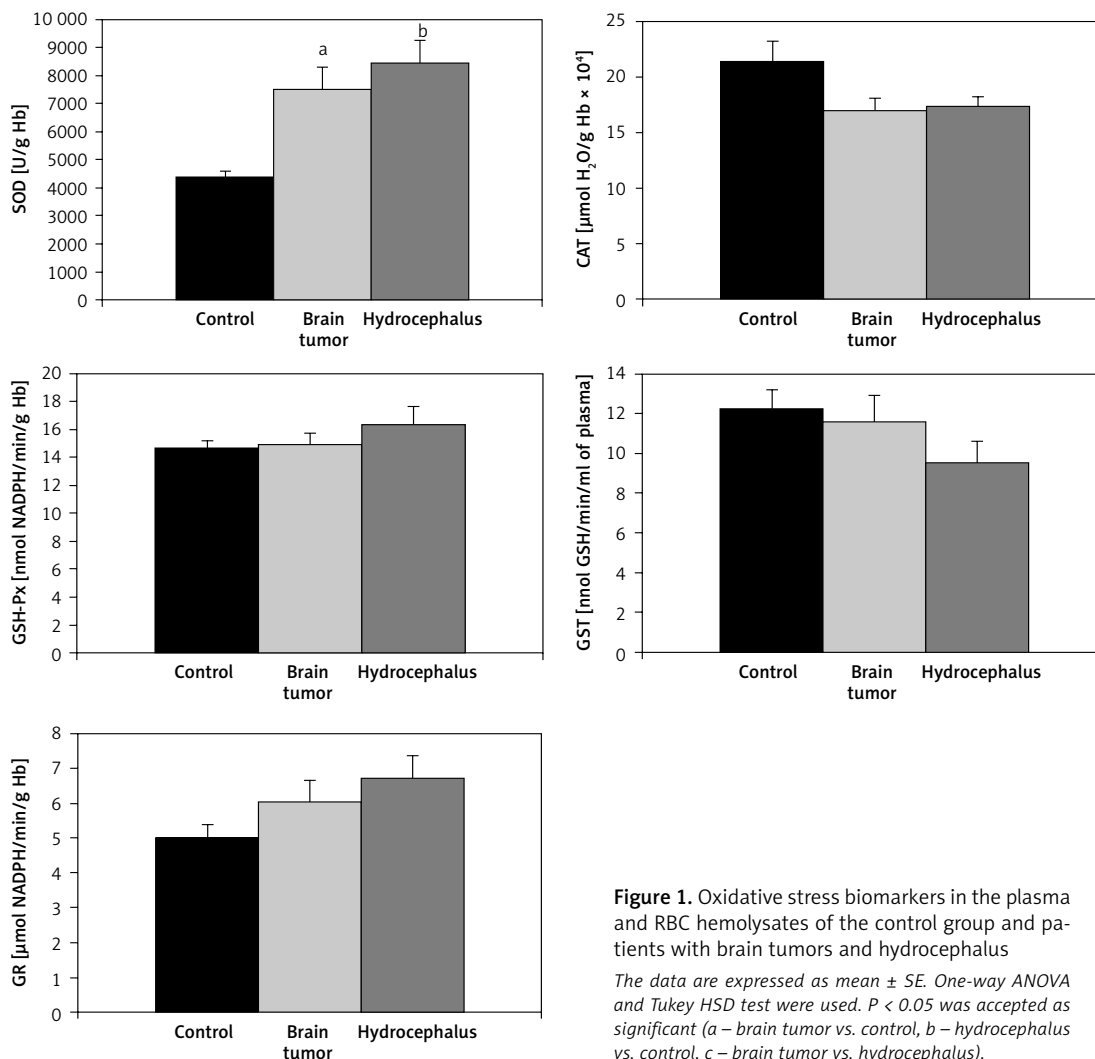
Ljiljana Vujotić, Siniša Matić, Slavica Borković-Mitić, Aleksandar Stojsavljević, Jelena Mutić, Vladimir Baščarević, Miloš Joković, Slađan Pavlović

Figure 1. Oxidative stress biomarkers in the plasma and RBC hemolysates of the control group and patients with brain tumors and hydrocephalus

The data are expressed as mean ± SE. One-way ANOVA and Tukey HSD test were used. P < 0.05 was accepted as significant (a – brain tumor vs. control, b – hydrocephalus vs. control, c – brain tumor vs. hydrocephalus).

groups of patients are presented in Figure 3 and indicate that factor 1 and factor 2 explain 100% of the total variance. Factor 1 (86%) clearly discriminates healthy (control) and sick (brain tumors and hydrocephalus) individuals. Factor 2 (14%) distinctly discriminates control and hydrocephalus groups from brain tumor patients.

Metal concentrations are presented in Table I. Ten metal ions (Mn, Ni, Co, Cu, Zn, As, Se, Cd, Hg, and Fe) were quantified in each sample of plasma and RBC hemolysates. Statistically significant differences in the concentration of metal ions in the plasma were obtained for the following metal ions: Mn, Ni, Co, Zn, As, Cd and Fe. Our results show lower concentrations of Mn, Ni and Zn in the plasma and Ni, Co and Fe in RBCs of brain tumor patients in comparison to the control group ($p <$ 0.05). In RBCs of brain tumor patients, higher concentrations of As, Se and Cd were observed. In patients with hydrocephalus we detected decreased concentrations of Ni, Co, Zn and Fe in the plasma, as well as Ni and Fe in RBCs also. Increased concentrations of As, Se and Cd were measured

in RBCs (As and Cd in the plasma) of same group of patients (Table I). Interestingly, there are higher concentrations of Cd in both plasma and RBCs of hydrocephalus patients when compared with patients with brain tumor ($p <$ 0.05).

Statistically significant Pearson's correlations between oxidative stress biomarkers as well as AChE activity and metal ions in the plasma and RBC hemolysate of patients with brain tumors and hydrocephalus, in relation to the controls are presented in Tables II and III. The data obtained for oxidative stress parameters determined in the plasma (GST, GSH, SH groups, LP) and for AChE activity were compared with the plasma metal concentrations, while data for oxidative stress parameters obtained in RBCs (CuZn SOD, CAT, GSH-Px and GR) were compared with metal concentrations detected in RBCs.

The Pearson's correlation coefficients of investigated oxidative stress parameters and metal ions in plasma are presented in Table II. In patients with brain tumors, positive correlations were obtained for Co and Cd with SH groups, as well as
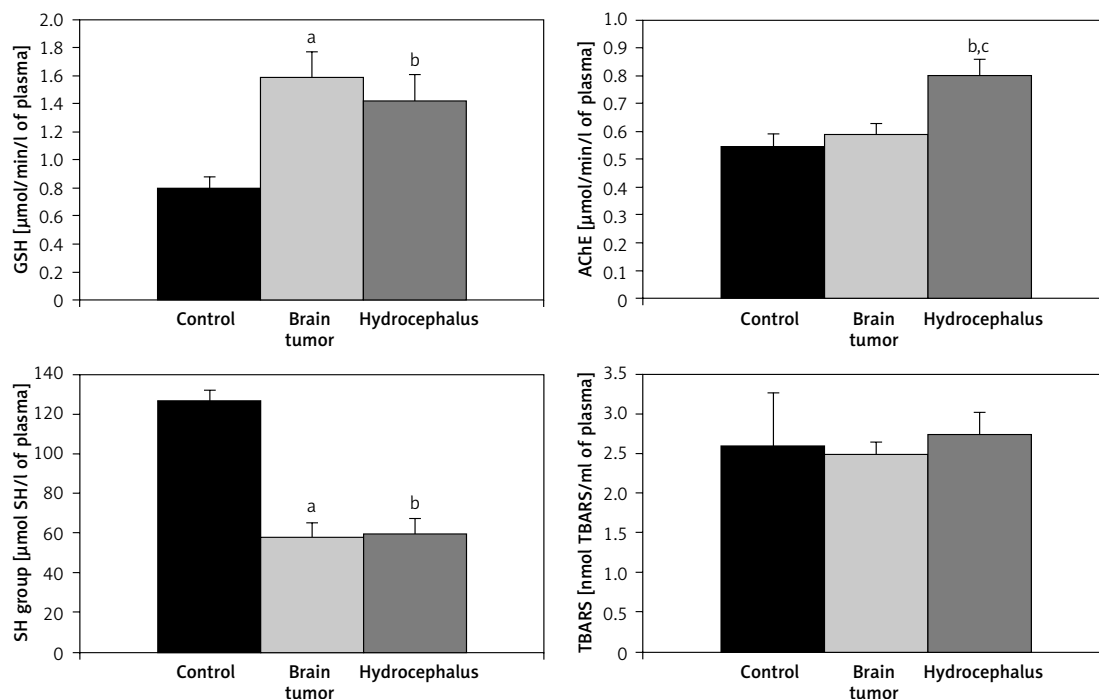
Figure 2. Oxidative stress biomarkers in the plasma and RBC hemolysates of the control group and patients with brain tumors and hydrocephalus

The data are expressed as mean ± SE. One-way ANOVA and Tukey HSD test were used. P < 0.05 was accepted as significant. (a – brain tumor vs. control, b – hydrocephalus vs. control, c – brain tumor vs. hydrocephalus).

for Hg and AChE activity. There are some positive correlations in the plasma of patients with hydrocephalus: Mn and Fe with GST, as well as Co and Zn with SH groups. Only one negative correlation was obtained for Co and AChE activity.

The obtained correlation coefficients of investigated parameters and metal ions in RBCs are presented in Table III. In patients with brain tumors, GSH-Px also correlated with Cu and Se. In the same group of patients, negative correlations were obtained between CuZn SOD and Hg, as well as between CAT and Cu and Se. In patients with hydrocephalus, SOD correlated positively with Se, and negatively with Ni, Cu, Cd and Hg.

Discussion

The complex series of cellular and molecular changes that occur through the development of cancer can be mediated by a diversity of endogenous and environmental stimuli. The human brain is especially vulnerable to free radical attack because of its high oxygen consumption and high concentrations of easily oxidizable polyunsaturated fatty acids. In addition, the brain's antioxidant capacity is lower compared with other organs, and thus the brain may be more susceptible to oxidative damage [22].

Increased oxidative stress was considered to have some role in the pathogenesis of various diseases [23]. Cancer in its complex pathology can
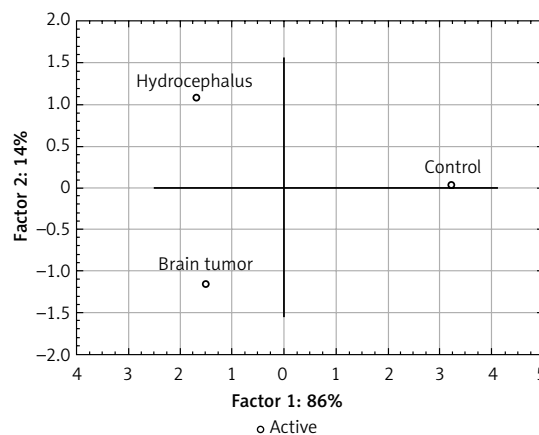

Figure 3. Principal component analysis (PCA) of all investigated oxidative stress biomarkers of each investigated group of individuals (Control, Brain tumor and Hydrocephalus) on the factor plane

Projection of the cases on the factor-plane (1 × 2). Cases with sum of cosine square ≥ 0.00.

be considered both a cause and a consequence of oxidative stress. Moreover, carcinogenic activities of substances that act as tumor promoters are associated with weakening of the cellular antioxidant defense system and decrease in some of its constituents [24]. The best solution in the process of brain tumor diagnosis could be readily available biomarkers that should be useful in the monitoring of the disease course, differential diagnosis and planning surgical intervention [25].

Ljiljana Vujotić, Siniša Matić, Slavica Borković-Mitić, Aleksandar Stojsavljević, Jelena Mutić, Vladimir Baščarević, Miloš Joković, Slađan Pavlović

Table I. Concentrations of selected metal ions (ng/g and µg/g) in the plasma and RBCs of the control group and patients with brain tumors and hydrocephalus

Parameter		Control	Brain tumor	Hydrocephalus
Mn [ng/g]	Plasma	7.14 ±6.45	3.55 ±2.36[a]	5.64 ±4.54
	RBCs	3.86 ±2.83	4.48 ±4.07	4.74 ±4.64
Ni [ng/g]	Plasma	6.75 ±2.35	4.44 ±2.56[a]	4.53 ±2.87[b]
	RBCs	3.12 ±1.02	1.73 ±1.27[a]	1.59 ±1.32[b]
Co [ng/g]	Plasma	0.80 ±0.56	0.58 ±0.53	0.42 ±0.55 [b]
	RBCs	0.31 ±0.27	0.20 ±0.47[a]	0.32 ±0.54
Cu [µg/g]	Plasma	1.22 ±0.38	1.15 ±0.27	1.08 ±0.24
	RBCs	0.10 ±0.02	0.10 ±0.04	0.09 ±0.02
Zn [µg/g]	Plasma	0.63 ±0.13	0.49 ±0.18[a]	0.57 ±0.17[b]
	RBCs	1.35 ±0.45	1.50 ±0.55	1.42 ±0.28
As [ng/g]	Plasma	1.54 ±0.58	1.77 ±0.55	1.91 ±0.79[b]
	RBCs	0.05 ±0.03	0.26 ±0.23[a]	0.21 ±0.19[b]
Se [ng/g]	Plasma	83.17 ±21.59	74.18 ±24.76	73.86 ±20.89
	RBCs	9.01 ±5.19	15.09 ±9.53[a]	14.24 ±6.65[b]
Cd [ng/g]	Plasma	0.01 ±0.00	0.02 ±0.02	0.05 ±0.02[bc]
	RBCs	0.01 ±0.00	0.10 ±0.27[a]	0.21 ±0.18[bc]
Hg [ng/g]	Plasma	0.31 ±0.27	0.42 ±0.26	0.45 ±0.27
	RBCs	0.37 ±0.30	0.47 ±0.34	0.46 ±0.28
Fe [µg/g]	Plasma	1.33 ±0.41	1.86 ±0.97	1.83 ±0.85[b]
	RBCs	196.84 ±29.93	149.02 ±36.91[a]	140.04 ±27.76[b]

The data are expressed as mean ± SD. P < 0.05 was accepted as significant (a – brain tumor vs. control, b – hydrocephalus vs. control, c – brain tumor vs. hydrocephalus).

Table II. Statistically significant correlations between biomarkers of oxidative stress, AChE activity, and metal concentrations in the plasma of patients with brain tumors and hydrocephalus

Investigated parameter	Metal ion	Brain tumor r	Hydrocephalus r
GST	Mn		0.46
GST	Co		
GST	Cu		
GST	Zn		
GST	As		
GST	Se		
GST	Cd		
GST	Fe		0.63
SH	Co	0.47	0.79
SH	Zn		0.62
SH	Se		
SH	Cd	0.45	
AChE	Co		−0.48
AChE	Hg	0.51	

Pearson's correlation coefficients (r) with respect to single metal concentration. A minimum significance level of p < 0.05 was accepted.

Table III. Statistically significant correlations between biomarkers of oxidative stress, AChE activity, and metal concentrations in RBCs of patients with brain tumors and hydrocephalus

Investigated parameter	Metal ion	Brain tumor r	Hydrocephalus r
GST	Mn		0.46
GST	Co		
GST	Cu		
GST	Zn		
GST	As		
GST	Se		
GST	Cd		
GST	Fe		0.63
SH	Co	0.47	0.79
SH	Zn		0.62
SH	Se		
SH	Cd	0.45	
AChE	Co		−0.48
AChE	Hg	0.51	

Pearson's correlation coefficients (r) with respect to single metal concentration. A minimum significance level of p < 0.05 was accepted.

In our study, RBCs' CuZn SOD activity was significantly higher in both groups of patients compared to the controls. The results of other authors show subnormal activities of SOD in tumors of the gastrointestinal tract multiple myeloma and endometrial cancer [26, 27]. A significant increase in CAT activity has been reported in various cancers such as gastric cancer [28] and carcinoma of the bladder [29]. Oxidative DNA damage in blood and other tissues was detected in various types of human carcinogenesis [30]. The GST is involved in detoxification of carcinogens and its activity increased significantly in cancer patients [31]. In smokers, the role of GST is crucial in modulating susceptibility to smoking-related lung cancer, oral cancer and chronic obstructive pulmonary disease [32]. It is also observed that GSH-Px and SOD activities decrease in the cancer patients during cancer development [33].

Kudo *et al.* found significant differences in the GSH concentration between glioblastomas and astrocytomas [34]. Radiosensitive tumors, such as multiple myeloma, germinoma and small-cell carcinoma, showed low GSH concentrations. In our study, glutathione concentration was significantly higher in brain tumor and hydrocephalus patients than in control individuals. The higher activity of CuZn SOD and concentration of GSH compared to controls could indicate higher oxidative stress in both groups of patients. The GSH system plays a very important role in the brain's defense. The GSH level was found to be significantly lower in glioblastomas compared to normal tissue and elevated only in meningioma [34]. A significant increase in all antioxidant enzyme activities and decrease in the GSH level were observed in some brain tumors [35].

Thiol-containing compounds such as SH groups are important components maintaining redox homeostasis in cells, tissues and biological fluids in an organism. Modification of membrane proteins by SH groups changes membrane permeability. Oxidative modification of SH groups in enzymes or in their coenzymes exerts an influence on enzymatic activity. Metal ions bind to SH groups, and the intracellular fate of essential and non-essential metal ions strongly depends on the level of thiol-containing molecules [36]. Another important feature of SH groups is that they serve as a marker of protein oxidation [37]. In our experiments, a significant decrease was noted in the concentration of SH groups.

In order to detect possible differences between investigated groups, we employed principal component analysis (PCA), which takes into account all the parameters examined. This analysis show clear separation between control individuals at the one hand, and patients with brain tumors and hydrocephalus on the other hand. At the same time, there is a clear separation between patients with brain tumors and hydrocephalus also.

While Fe, Cu and Co undergo redox cycling reactions, for a second group of metal ions, Hg, Cd and Ni, the primary route for realization of their toxicity is depletion of glutathione and binding to sulfhydryl groups of proteins. Arsenic (As) is thought to bind directly to critical thiols. However, other mechanisms involving formation of hydrogen peroxide under physiological conditions have also been proposed [38].

In the study of Kuo *et al.* [39], Zn, Fe and Se concentrations were found to be significantly lower in cancer patients, while Cu concentrations were found to be either elevated or significantly elevated when compared to age-matched samples of normal tissues. Transition metal ions such as Mn and Fe have been found to be present in significantly lower concentrations in some tumors. Recent studies imply a low antioxidant status and enhanced oxidative stress in cancer patients, even before chemotherapy starts.

High levels of oxidative stress result in peroxidation of membrane lipids with the generation of peroxides that can decompose to multiple mutagenic carbonyl products. Malondialdehyde (MDA) is a well-characterized lipid peroxidation end product. MDA is considered to be mutagenic and carcinogenic. The level of MDA reflects the extent of lipid peroxidation [40]. Elevated levels of lipid peroxidation products support the hypothesis that cancer cells produce a large amount of free radicals and that there exists a relationship between free radical activity and malignancy [41]. In our study, there were no statistically significant differences in lipid peroxidation between the studied groups.

In our study a significant increase in AChE activity was observed in hydrocephalus when compared to both control and brain tumor groups. The absence of ChE inhibition could be a consequence of chronic contaminant exposure and of similar concentrations of most of the investigated metal ions. The ChE family includes acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE), also known as pseudocholinesterase [42]. Cholinesterases play an important role in nerve impulse transmission and are generally used as indicators of neurotoxicity [43]. There is evidence that heavy metal ions can alter ChE activities, and one of the possible mechanisms is through the ability of metal ions to cause diverse post-translational modifications of ChE proteins [44].

From the presented results, it can be concluded that brain tumor and hydrocephalus patients have increased oxidative stress in the plasma and RBCs. The results of the present study support the concept of involvement of free radicals in the development of intracranial tumors and

Ljiljana Vujotić, Siniša Matić, Slavica Borković-Mitić, Aleksandar Stojsavljević, Jelena Mutić, Vladimir Baščarević, Miloš Joković, Slađan Pavlović

hydrocephalus. Increases in the activity of CuZn SOD and GSH concentration, and decreases of CAT and GST activities, as well as concentration of SH groups, may be the major factors responsible for oxidative stress. In both groups of patients we observed decreased concentrations of Ni, Co, Zn and Fe (and Mn in brain tumor patients) and increased concentrations of As, Se and Cd in the blood. We also observed higher concentration of Cd in both plasma and RBCs of hydrocephalus patients compared to the patients with brain tumor. There is a strong correlation between some metal concentrations and certain oxidative stress biomarkers in the blood of patients, which supports our hypothesis, but the observed trend should be further investigated.

Acknowledgments

Conflict of interest

The authors declare no conflict of interest.

References

1. Halliwell B, Gutteridge JMC. Free Radicals in Biology and Medicine. 4th ed. Oxford University Press Inc, New York 2007.
2. American Medical Association. In: The American Medical Association's/Encyclopedia of medicine. Charles B (ed.). Random House, Clayman 1989; 396-752.
3. Westbrook GL, Mayer ML. Micromolar concentrations of Zn antagonize NMDA and GABA response of hippocampal neurons. Nature 1987; 328: 640-43.
4. Takeda A. Zinc homeostasis and functions of zinc in the brain. Biometals 2001; 14: 343-51.
5. Margaill I, Plotkine M, Lerouet D. Antioxidant strategies in the treatment of stroke. Free Radic Biol Med 2005; 39: 429-43.
6. Socci DJ, Bjugstad KB, Jones HC, Pattisapu JV, Arendash GW. Evidence that oxidative stress is associated with the pathophysiology of inherited hydrocephalus in the H-Tx rat model. Exp Neurol 1999; 155: 109-17.
7. Nuss JI, McCarl RL, Mulay IL, Mulay LN. Copper and free radical accumulation in liver of calves with inherited hydrocephalus. Am J Vet Res 1967; 28: 1909-13.
8. Louis DN, Ohgaki H, Wiestler OD, Cavenee WK. WHO Classification of Tumours of the Central Nervous System. 4th edn. IARC, Lyon 2007.
9. Fazio F, Cecchini S, Faggio C, Caputo AR, Piccione G. Stability of oxidative stress biomarkers in flathead mullet, Mugil cephalus, serum during short-term storage. Ecol Indic 2014; 46: 188-92.
10. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J Biol Chem 1974; 249: 7130-9.
11. Griffith OW. Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. Anal Biochem 1980; 106: 207-12.
12. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys 1959; 82: 70-7.
13. Ellman GL, Courtney KD, Andres VJR, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 1961; 7: 88-95.
14. Ohkawa H, Okishi N, Yogi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979; 95: 351-8.
15. Drabkin D, Austin H. Spectrophotometric studies preparations from washed blood cells. J Biol Chem 1935; 112: 51-65.
16. Tsuchihashi M. Zür Kenntnis der Blut katalase. Biochem Z 1923; 140: 65-74.
17. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 1972; 247: 3170-5.
18. Beutler E. Catalase. In: Red Cell Metabolism, a Manual of Biochemical Methods. Beutler E (ed.). Grune and Stratton Inc, New York 1982; 105-6.
19. McCord JM, Fridovich I. Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). J Biol Chem 1969; 244: 6049-55.
20. Maral J, Puget K, Michelson AM. Comparative study of superoxide dismutase, catalase and glutathione peroxidase levels in erythrocytes of different animals. Biochem Biophys Res Commun 1977; 77: 1525-35.
21. Glatzle D, Vuilleumier JP, Weber F, Decker K. Glutathione reductase test with whole blood, a convenient procedure for the assessment of the riboflavin status in humans. Experientia 1974; 30: 665-7.
22. Kelly PJ, Morrow JD, Ning M, et al. Oxidative stress and matrix metalloproteinase-9 in acute ischemic stroke: the biomarker evaluation for antioxidant therapies in stroke (BEAT-stroke) study. Stroke 2008; 39: 100-4.
23. Arkadiusz Z, Stepniak J, Wojciechowska-Durczynska K, Krawczyk-Rusiecka K, Lewinski A, Karbownik-Lewinska M. Relationship between urine lipid peroxidation, anthropometric parameters and parameters associated with goitre formation in school-age children. Arch Med Sci 2018; 14: 30-7.
24. Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Radic Biol Med 1991; 11: 81-128.
25. Jelski W, Laniewska-Dunaj M, Orywal K, Kochanowicz J, Rutkowski R, Szmitkowski M. The diagnostic value of alcohol dehydrogenase (ADH) isoenzymes and aldehyde dehydrogenase (ALDH) measurement in the sera of patients with brain tumor. Arch Med Sci 2017; 13: 346-52.
26. Chevaris S, Andial T, Benke K, Strenger A. Free radical reactions and cancer. Voprosy Meditsin Khim 1992; 38: 4-5.
27. Zima T, Spi C, Ka I, Stipek S. Lipid peroxidation and activity of antioxidative enzymes in patients with multiple myeloma. Casopis Lekaru Ceskych 1996; 135: 14-7.
28. Beno I, Volkovov AK, Staruchov AM. Gastric mucosal antioxidant activity in patients at increased risk of gastric cancer. Neoplasma 1993; 40: 315-9.
29. Singh SV, Xu BH, Tkalcevic GT, Gupta V, Roberts B, Quiz P. Glutathione-linked detoxification pathway in normal and malignant human bladder tissue. Cancer Lett 1994; 77: 15-24.
30. Yano T, Shoji F, Baba H, et al. Significance of the urinary 8-OHdG level as an oxidative stress marker in lung cancer patients. Lung Canc 2009; 63: 111-4.
31. Beevi SS, Rasheed MH, Geetha A. Evidence of oxidative and nitrosative stress in patients with squamous cell carcinoma. Clin Chim Acta 2007; 375: 119-23.
32. Yanbaeva DG, Wouters EF, Dentener MA, Spruit MA, Reynaert NL. Association of glutathione-S-transferase ome-

ga haplotypes with susceptibility to chronic obstructive pulmonary disease. Free Radic Res 2009; 43: 738-43.

33. Fiaschi AI, Cozzolino A, Ruggiero G, Giorgi G. Glutathione, ascorbic acid and antioxidant enzymes in the tumor tissue and blood of patients with oral squamous cell carcinoma. Eur Rev Med Pharmacol Sci 2005; 9: 361-7.

34. Kudo H, Mio T, Kokunai T, Tamaki N, Sumino K, Matsumoto S. Quantitative analysis of glutathione in human brain tumors. J Neurosurg 1990; 72: 610-5.

35. Dudek H, Farbiszewski R, Rydzewska M, Michno T, Kozłowski A. Evaluation of antioxidant enzymes activity and concentration of non-enzymatic antioxidants in human brain tumours. Wiad Lek (Warsaw) 2004; 57: 16-9.

36. Eaton DL, Stacey NH, Wong KL, Klaassen CD. Dose–response effects of various metal ions on rat liver, metallothionein, glutathione, heme oxygenase and cytochrome P-450. Toxicol Appl Pharmacol 1980; 55: 393-402.

37. Labieniec M, Gabryelak T. Antioxidative and oxidative changes in digestive gland cells of freshwater mussels Unio tumidus caused by selected phenolic compounds in the presence of H_2O_2 or Cu^{2+} ions. Toxicol Vitro 2007; 21: 146-56.

38. Valko M, Morris H, Cronin MTD. Metals, toxicity and oxidative stress. Curr Med Chem 2005; 12: 1161-208.

39. Kuo HW, Chen SF, Wu CC, Chen DR, Lee JH. Serum and tissue trace elements in patients with breast cancer in Taiwan. Biol Trace Elem Res 2002; 89: 1-11.

40. Zhang Y, Chen Hsu T, Santella RM. Immunohistochemical detection of malondialdehyde-DNA adducts in human oral mucosa cells. Carcinogenesis 2002; 23: 207-11.

41. Dormandy TI. An approach to free radicals. Lancet 1983; 322: 1010-14.

42. Çokuğraş AN. Butyrylcholinesterase: structure and physiological importance. Turk J Biochem 2003; 28: 54-61.

43. Triquet CA, Leguille CC, Mouneyrac C. Biomarkers of defense, tolerance, and ecological consequences. In: Ecological Biomarkers, Indicators of Ecotoxicological Effects. Triquet CA, Amiard JC, Rainbow PS (eds.). CRC Press Inc, Boca Raton 2013; 45-75.

44. Richetti SK, Rosemberg DB, Ventura-Lima J, Monserrat JM, Bogo MR, Bonan CD. Acetylcholinesterase activity and antioxidant capacity of zebrafish brain is altered by heavy metal exposure. Neurotoxicology 2011; 32: 116-22.