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The relationship between rehabilitation outcomes and extracellular thiol-disulphide and intracellular oxidized-reduced glutathione homeostasis in patients with subacute stroke

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Abstract

Background & Objective: To evaluate the relationship between the clinical outcome of subacute stroke patients and extracellular thiol-disulphide (SH-SS) and intracellular oxidized-reduced glutathione (GSSG-GSH) homeostasis and the effect of the rehabilitation program on these homeostasis. *Methods:* In this prospective observational study, outcome assessments (National Institutes of Health Stroke Scale Scores (NIHSS), modified Rankin Scale (mRS), and Barthel Daily Living Activities Index (BI)) and SH-SS and GSSG-GSH homeostasis parameters were investigated from 42 patients with subacute stroke before and after 4-week rehabilitation treatment protocol. Also, SH-SS and GSSG-GSH homeostasis parameters were measured from 35 healthy volunteers. *Results:* SS/SH and GSSG/GSH ratios were significantly higher in the patient group at baseline and post-rehabilitation than the control group ($p<0.05$). SS/SH ratio, GSSG/GSH ratio, NIHSS and mRS values significantly decreased with the rehabilitation ($p<0.05$). The baseline SS/SH and GSSG/GSH ratios were correlated with baseline and post-rehabilitation NIHSS, mRS and BI scores ($p<0.05$).
Conclusions: The better outcomes of patients with higher baseline antioxidant thiol groups suggest that active thiol groups may play a role in achieving better outcomes in subacute stroke. In addition, rehabilitation treatment increased clinical scores and additionally shifted intracellular and extracellular thiol groups to the antioxidant side.

Keywords: Thiol, disulfide, glutathione, stroke, stroke rehabilitation, oxidative stress

INTRODUCTION

The disability-adjusted life years (DALY) due to stroke, which is the second most common cause of all deaths in the world, is 116.4 million.[1,2] Despite continued advances in patient care, stroke remains one of the leading causes of disease burden worldwide.[1,3] In stroke rehabilitation, it is important to determine the causes of post-stroke damage, to determine the underlying mechanisms of neuro-biological recovery, and to increase the understanding of the factors that are measured early after stroke and predict the final outcome. A reliable and consistent biomarker that can be measured with serum, plasma or whole blood in the follow-up may be useful in guiding treatment and predicting response to treatment. Neurons are highly susceptible to oxidative damage due to their high metabolic and low regeneration rates.[4] Oxidative stress plays a role in both the pathophysiology of stroke and the mechanisms of post-stroke complications.[5] Therefore, it is thought that determining the levels of oxidative stress markers and antioxidant defense molecules and investigating their effects on clinical recovery may have an important place in the follow-up and rehabilitation planning of patients with stroke.[2]

Oxidative stress is defined as an imbalance between oxidants and antioxidants in favor of oxidants, which causes deterioration and/or molecular damage in a cell, tissue, or organ.[6] Molecules containing thiol groups (SH) undergo an oxidation reaction with oxidant molecules to form molecules containing reversible dynamic

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disulfide bonds (SS).[7] Thus, while SH level in the environment is an antioxidant marker, the level of SS formed is considered as an indicator of oxidative stress marker.[8] A large part of the plasma or serum thiol pool is formed by the SH groups of proteins such as albumin.[9] However, the majority of the thiol pool of the intracellular is composed of glutathione, which is the major and the most abundant antioxidant molecule in the cell and brain.[10] Serum SH-SS and intracellular oxidized-reduced glutathione (GSSG-GSH) balances are markers indicating extracellular and intracellular oxidative stress and antioxidant capacity.[8] Besides removing ROS, glutathione is renewing other antioxidant molecules, and repairing oxidized cell contents.[11] Thus, low glutathione levels are associated with decreased growth, differentiation, and increased apoptosis in neuron cells.[11]

Extracellular SH-SS balance was shown to be disrupted in acute stroke.[12,13] Thiol groups have many intracellular functions. In addition to being an important component of antioxidant defense, thiol groups (SH) are critical in enzyme function by being located in the active sites of enzymes, folding and functionality of proteins, detoxification, regulation of transcription factors, signal transduction, apoptosis, and cellular stimulation mechanisms.[9,14] It has been stated that the intracellular and extracellular thiol balances have differences.[15] So, evaluating extracellular thiols without intracellular thiols could not provide direct information about intracellular thiols. To the best of our knowledge there is no study in which both extracellular SH-SS and intracellular GSSG-GSH balances were studied together or in which the relationship of these balances with rehabilitation outcomes were investigated in the subacute phase of stroke. Therefore, by studying both compartments' thiols together, it will be possible to obtain direct information about the thiol balances of both compartments. In this way, the relationship between the intracellular and extracellular thiols and the disease will be better understood.

In this study, we aimed to compare the SH-SS and GSSG-GSH balances in subacute stroke patients with healthy volunteers, to evaluate the relationship among these balances with the severity-related and the functional status-related outcomes, to determine the predictive value of these balances' parameters on the severity and functional status-related outcomes after 4 weeks of rehabilitation intervention in patients with subacute stroke.

METHODS

In this prospective observational study, patients with stroke who applied to AIBU Izzet Baysal Physical Therapy and Rehabilitation Training and Research Hospital for physiotherapy and rehabilitation and healthy volunteers had no known disease and no findings on physical examination were included. This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the BAIBU Clinical Research Ethics Committee based on the decision (decision number: 2020/68). Written informed consent was obtained from all of the individuals.

Inclusion criteria were as follows: Adult patients (>18-year-old) with the first-ever stroke confirmed by computed tomography or magnetic resonance imaging reports and admitted to the hospital for conventional rehabilitation treatment at 1 to 6 months after stroke onset, and adult healthy volunteers. Exclusion criteria were as follows: Having an autoimmune disease, infection, tumor, heart failure, kidney or liver dysfunction, acute neurological disease other than stroke (such as head trauma, brain abscess, brain tumor, migraine attack, seizure), inability to adapt to study (such as patients with psychiatric disorders, inability to give informed consent), having a rehabilitation treatment history, taking antioxidant supplements and/or not completing the 4-week rehabilitation treatment protocol (patients who want to transfer another center, patients who referred to another hospital due to complications such as myocardial infarction, pulmonary embolism, infection and/or deterioration of general health).

Patients received conventional rehabilitation programs, including passive and active range of motion exercises, in-bed movements, sitting and transfer training, balance and mobility exercises, and strengthening exercises, administered by a licensed physiotherapist for 45 minutes/day 5 days/week during the 4-week study period. Functional outcomes were administered to all patients before (t0) and after the rehabilitation intervention (t1) by the one rater (TA). The 4-week rehabilitation program is a treatment protocol that is routinely applied in our center. At the end of this period, the patients are evaluated in terms of the suitability of continuing the rehabilitation program at home. Therefore, 4-week rehabilitation program was set as the study period.

Blood samples

Blood samples were taken from patients into serum separating tubes and ethylenediaminetetraacetic acid (EDTA) containing tubes (BD Vacutainer; Becton Dickinson, Heidelberg, Germany) at each t_0 and t_1 and from healthy volunteers once by an experienced phlebotomist between 8:00 and 10:00 am. Samples in EDTA anticoagulant-containing tubes were immediately centrifuged and washed thrice with 0.9% NaCl and lysed with deionized water. Then, 1-part 20% w/v TCA solution was mixed with 3-part lysed erythrocyte samples to precipitate proteins. After centrifugation of these mixed samples, supernatants were obtained and were stored at –80°C. After waiting for the clotting process for 15 minutes, the serum separating tubes were centrifuged, and the obtained serum samples were stored at –80°C. All supernatant and serum samples were thawed on the day of analysis, and the determination of parameters was made with an automated analyzer (Abbott Architect c8000, Abbott Diagnostics, Chicago, IL, USA).

Measurement of serum SH-SS homeostasis parameters

SH-SS balance parameters were determined using the method described by Erel and Neselioglu (7) from serum samples. Briefly, two parallel measurements were made to determine the SH-SS balance parameters. First, the native SH (NT) level in the serum was measured with DTNB. Secondly, after the SSs in the serum were reduced to SHs with $NaBH_4$, they were measured again after being used to remove the remnant $NaBH_4$ by formaldehyde. The value obtained from this second measurement was expressed as total thiol (TT) as it contains the SH levels from the reduction of the SS and NT. Half of the difference between TT and NT values was calculated as SS levels. Results were expressed as μmol/L. Also, SS/SH percent ratio was calculated.

Measurement of serum GSSG-GSH homeostasis parameters

GSSG-GSH balance parameter levels were determined from supernatant samples by the method described by Alisik *et al*.[16] GSH levels of supernatant samples were determined using Ellman's reagent (500 mM Tris solution, pH: 8.2). After that, the first GSSG in supernatant samples were reduced with $NaBH_4$ and NaOH to form GSH. The HCl solution was added to remove the excess $NaBH_4$ to prevent extra-reduction of DTNB molecules and re-oxidation of GSH molecules. After this process, another GSH measurement was made with DTNB which constitutes the sum of GSHs coming from the reduction of GSSGs and native reduced GSH and the results were expressed as total GSH (TGSH). GSH content was subtracted from the TGSH content and divided by two equals to the GSSG amount. Results were expressed as μmol/L. GSSG/GSH percent ratios were also calculated.

Functional outcomes

The National Institutes of Health Stroke Scale: NIHSS, defined by the American National Institute of Health, is used to quantitatively determine the severity of the disease neurologically.[17] NIHSS is a scale that examines neurological functions in patients with stroke and gives an idea about the long-term prognosis. It is a neurological assessment scale that includes 15 items in 11 categories and provides information about the level of consciousness, visual assessment, motor functions, sensory neglect, and cerebellar functions. High scores in NIHSS indicate more severe neurological damage, and NIHSS is scored between 0 and 42 points.

modified Rankin Scale: mRS is used to evaluate the degree of functional independence of patients.[17] In this scale, an evaluation is made between 0 and 6 points and 0 points indicate complete independence, while 6 points mean death.

Barthel Activities of Daily Living Index: The ability of patients to perform basic daily life activities was evaluated using the BI.[18] BI consists of 10 items and is a scale evaluating over a total of 100 points. High scores in BI mean the ability to perform more basic daily life activities.

Statistical analysis

Statistical analysis was performed by using the SPSS statistical software for Windows, version 20, released in 2011 (IBM, Armonk, NY, USA). The Kolmogorov–Smirnov test was used to evaluate the normality distribution assumption of parameters. The descriptive statistics were expressed as means±standard deviations (SD) for variables with normal distributions, medians (1^{st} to 3^{rd} quartile value (Q1 to Q3)) for non-normally distributed variables, and the number of cases (percentages) for categorical variables. Two group comparisons were made with Student's t test for normally distributed independent parameters and with Mann-Whitney U test for

non-parametric parameters. The changes in levels of parameters of balances and functional outcomes were examined with paired t test in normally distributed data and with Wilcoxon test in non-parametrically distributed data. Categorical variables were compared with the Pearson Chi-square test or Fisher's exact test, which was appropriate. In correlation analysis, the Pearson correlation test was used for parametrically distributed quantitative variables, and the Spearman correlation test was used for nonparametric numerical data. The significance level was set at $p<0.05$.

RESULTS

A total of 63 patients and 35 healthy volunteers who met the inclusion and exclusion criteria were included in the study. During the study, 21 patients were excluded. Fifteen of them wanted to continue their treatment at their homes before the end of the treatment protocol, 3 of them developed infection and 3 of them were referred to another hospital because of complications (1 re-stroke, 1 myocardial infarction, and 1 pulmonary embolism). Demographic and clinical parameters of 42 patients with ischemic subacute stroke and 35 healthy control volunteers were shown in Table 1.

NIHSS, mRS, and BI significantly improved after treatment ($p<0.05$ for all) (Table 2). Comparing intracellular and extracellular thiol levels between groups, intracellular GSSG/GSH percent ratio, and extracellular SS/SH percent ratio values were higher in stroke patients than healthy participants at t0 and t1 ($p<0.05$ for all). These ratios significantly decreased at t1 compared to t0 (Table 3).

The relationship of pre-rehabilitation SH-SS and GSSG-GSH balances, the balance parameters with NIHSS, BI, and mRS scores at t0 and t1 were demonstrated in Table 4.

DISCUSSION

In this study, it was observed that intracellular GSH-GSSG balance and extracellular SH-SS balance deteriorated towards the oxidant side, associated with worse outcomes determined by NIHSS, BI, and mRS in patients with subacute stroke. In addition, these balance and outcome scores were significantly improved with the rehabilitation program. Lower GSSG/GSH and SS/SH percent ratios were also shown to be associated with favorable outcomes assessed after 4 weeks of the rehabilitation program.

In two different studies evaluating the SH-SS balance in patients with acute stroke, they revealed a decrease in SH levels with an insignificant increase in the SS level.[12,13] In another study in which only the SH component of the SH-SS balance was examined, it was shown that SH levels decreased in patients after stroke.[19] Tsai *et al*. showed that SH levels decreased in patients with acute ischemic stroke compared to healthy volunteers, and they found that SH levels were lower in patients with large vessel disease than those with small vessel disease. They also showed

Table 1: Demographic and clinical parameters of stroke patient and healthy controls

		Patient (n=42)	Control (n=35)	P value
Age, years	**Md (Q1 to Q3)**	62 (54.8 to 68.3)	61 (55 to 64)	0.116*
Gender	**Male, n (%)**	22 (52.4%)	16 (45.7%)	0.560**
	Female, n (%)	20 (47.6%)	19 (54.3%)	
Hemiplegic side	**Right, n (%)**	23 (54.8%)		
	Left, n (%)	19 (45.2%)		
Dominant side	**Right, n (%)**	40 (95.2%)		
	Left, n (%)	2 (4.8%)		
Chronic Disease	**Diabetes mellitus, n (%)**	9 (21.4%)		
	Hypertension, n (%)	15 (35.7%)		
	Coronary artery disease, n (%)	6 (14.3%)		
Smoker	**n (%)**	8 (19%)		
Alcohol user	**n (%)**	3 (7.1%)		

Md (Q1 to Q3): median (1st to 3rd quartile); $\bar{x}$±SD: mean ± standard deviation; *: Mann-Whitney U test; **: Pearson's chi-squared tes

Table 2: Comparison of changes in clinical outcome measures of patients before (t0) and after (t1) rehabilitation

	t0	t1	Difference (t1-t0)	p value*
NIHSS	13 (9 to 16)	10 (7 to 11)	-3 (-4 to -2)	<0.001
BI	47(34 to 64)	69 (57.8 to 80.3)	18 (13.0 to 22.5)	<0.001
mRS	4 (3 to 4)	3 (2 to 3)	-1 (-1 to -1)	<0.001

Data shown as median (1st -3rd quartile). NIHSS: National Institutes of Health Stroke Scale, BI: Barthel Index, mRS: modified Rankin Scale, *: Wilcoxon test

that low SH levels were lower in patients with a worse stroke outcome 3 months later.[20] Similarly, we found that pretreatment SH levels were negatively correlated with posttreatment NIHHS and positively correlated with posttreatment BI scores. These findings suggest that low antioxidant SH levels contribute to further neurologic damage in particular cases. In addition, we found that pretreatment intracellular oxidized glutathione levels were more associated with worse outcome scores of NIHSS, BI and mRS than extracellular SS levels. These findings suggest that intracellular thiol dependent oxidative stress marker, GSSG, is better than extracellular oxidative stress marker, SS, in predicting post-treatment outcomes. Also, we think that high sensitivity to oxidative damage due to high metabolic and low regeneration rates of neurons[4] may be the reason for these relationships and/or intracellular glutathione levels of whole blood may better reflect the situation within the neuron cells.

The relationship between stroke severity and clinical outcome scores and oxidative stress markers has been demonstrated by various studies. Leinonen et al. revealed that the serum SH groups evaluated on the 2nd and 7th days were positively correlated with the better outcome scores which were evaluated by the average NIHSS and BI

Table 3: Comparison of the changes between pre-rehabilitation (t0) and post-rehabilitation (t1) in the extracellular thiol-disulfide and oxidized-reduced glutathione balances' parameters of the patients and comparison of these values with the control group.

		Patient (n=42)		Control (n=35)	p values		
		t0	t1		t0 vs t1	t0 vs Control	t1 vs Control
Extracellular	**Total Thiol, µmol/L**	298.5±45.5	345.8±57.0	371±65.3	<0.001*	<0.001†	0.075†
	Native Thiol (SH), µmol/L	242.1±46.0	295.3±58.7	339.7±62.6	<0.001*	<0.001†	0.002†
	Disulfide (SS), µmol/L	28.18±4.53	25.24±5.66	15.63±3.1	0.008*	<0.001†	<0.001†
	SS/SH ratio, %	12.1±3.4	9.07±3.33	4.7±1.0	<0.001*	<0.001†	<0.001†
Intracellular	**Total Glutathione, µmol/L**	892.0 (861.9-944.2)	951.4 (905.7-985.2)	1003.7 (816.3-1128.3)	<0.001‡	0.058§	0.208§
	Reducted Glutathione (GSH), µmol/L	686.1 (669.1-733.7)	765.0 (723.0-818.7)	932.7 (736.4-1052.5)	<0.001‡	<0.001§	0.001§
	Oxidized Glutathione (GSSG), µmol/L	101.3 (87.3-109.7)	90.0 (80.2-99.7)	33.3 (21.8-59)	<0.001‡	<0.001§	<0.001§
	GSSG/GSH ratio, %	14.1 (11.7-16.1)	11.3 (9.91-13.3)	4.8 (2.1-7.6)	<0.001‡	<0.001§	<0.001§

Non-parametric continuous variables were expressed as median (1st-3rd quartile) and parametric continuous variables are expressed as mean ± standard deviation. *: paired samples t-test, †: Student's t test, ‡: Wilcoxon test, §: Mann-Whitney U test.

Table 4: The relationship of pre-rehabilitation extracellular thiol-disulfide and intracellular oxidized-reduced glutathione balances' parameters with pre-rehabilitation (t0) and post-rehabilitation (t1) NIHSS, BI, and mRS scores

			t0			*t1*		
			NIHSS	**BI**	**mRS**	**NIHSS**	**BI**	**mRS**
Extracellular	Total Thiol, µmol/L	rho	-0.477**	0.282	-0.301	-0.460**	0.292	-0.313*
		p	0.001	0.070	0.053	0.002	0.060	0.044
	Native Thiol (SH), µmol/L	rho	-0.498**	0.322*	-0.300	-0.492**	0.333*	-0.313*
		p	0.001	0.038	0.053	0.001	0.031	0.043
	Disulfide (SS), µmol/L	rho	0.240	-0.257	0.154	0.309*	-0.253	0.130
		p	0.126	0.101	0.331	0.047	0.106	0.412
	SS/SH ratio, %	rho	0.512**	-0.410**	0.315*	0.553**	-0.387*	0.333*
		p	0.001	0.007	0.042	<0.001	0.011	0.031
Intracellular	Total Glutathione, µmol/L	rho	0.003	0.103	0.095	-0.006	0.108	0.131
		p	0.986	0.514	0.549	0.970	0.496	0.407
	Reducted Glutathione (GSH), µmol/L	rho	-0.404**	0.529**	-0.302	-0.443**	0.535**	-0.314*
		p	0.008	<0.001	0.052	0.003	<0.001	0.043
	Oxidized Glutathione (GSSG), µmol/L	rho	0.476**	-0.463**	0.461**	0.550**	-0.482**	0.566**
		p	0.001	0.002	0.002	<0.001	0.001	<0.001
	GSSG/GSH ratio, %	rho	0.514**	-0.565**	0.481**	0.588**	-0.576**	0.563**
		p	<0.001	<0.001	0.001	<0.001	<0.001	<0.001

NIHSS: National Institutes of Health Stroke Scale, BI: Barthel Index, mRS: modified Rankin Scale, rho: Spearman's rank correlation coefficient

scores on the 1st day, 3rd month, 6th month, and 12th months.[21] Bektas *et al*. showed that low SH levels in acute stroke patients were associated with severe disease assessed by NIHSS.[13] Similarly, Musumeci *et al*. found that serum SH levels were lower in patients with severe stroke compared to those without severe cases.[22] Manolescu et al. showed that SH levels of patients who had a stroke in the last 90 days were significantly lower than the control group, but that SH levels did not differ significantly from the control group after the 2-week rehabilitation program.[19] Also, they showed that there was a significant increase in BI values with treatment and showed that post-treatment BI values had a positive relationship with non-protein SH levels.[19] At the same time, they found that the protein carbonyl groups, which are among the oxidant markers, significantly decreased with treatment.[19] Similarly, in our study, the correlation between worse scores in NIHSS, BI, and mRS scales and low antioxidant levels and high oxidant parameters support the idea that the deterioration in SH-SS and GSSG-GSH balances may be one of the mechanisms related to disease severity. However, the association of pretreatment SS / SH and GSSG / GSH ratios with post-treatment NIHSS, BI, and mRS scores suggest that these thiol redox systems may be used as prognostic markers.

Rehabilitation programs can provide functional improvement in stroke patients. It is known that exercise increases oxidative stress depending on its duration and intensity, as well as, physical therapy exercises increase oxidative stress.[23] However, it has been shown that continuous and regular exercise provides an increase in antioxidant capacity and a decrease in the level of oxidant molecule production due to exercise.[23,24] Kihoin *et al*.[23] showed that the patients having stroke who exercised for 1 hour a day, 6 days a week and for a total of 56 days had a significant increase in antioxidant levels and a significant decrease in oxidant levels, but this was not seen in the group who did not exercise. Thus, it shows that exercise program increases antioxidant capacity and resistance to oxidative stress. In another study, rehabilitation for 6 weeks in stroke patients, who were taken into rehabilitation on the 7th day after stroke and who have high total peroxide and nitrite oxide (NO) metabolites and low TAC,

copper/zinc-dependent superoxide dismutase (SOD), and serum urate levels compared to the control group provided significant improvement in NIHSS and BI scores. In addition, although the improvement in mRS scores were not significant, they reported a significant increase in antioxidants and a significant decrease in oxidants.[25] The 4-week rehabilitation program resulted in a significant improvement in Fugl-Meyer Assessment (FMA) and Medical Research Council (MRC) scales and a significant decrease in 8-hydroxy-2′-deoxyguanosine (8-OHdG), a DNA oxidation marker, in 61 post-stroke patients It was also suggested that 8-OHdG scores could be a predictive marker since 8-OHdG values at admission were associated with post-treatment FMA and MRC scores.[26] Similarly, in our study, it was shown that the rehabilitation program provided both clinical and functional improvement and increased antioxidant capacity along with a decrease in oxidative stress. This improvement may be related to the fact that continuous and regular exercise increases the production of antioxidant enzymes (manganese-dependent SOD, GSH peroxidase and Glutamate Cysteine Ligase, etc.).[27] Thus, the relationship between the improvement in stroke assessment scores and the reduction of oxidative stress can be explained by the reduction of both disease-related and exercise-induced oxidative stress as a result of increasing the antioxidant capacity of continuous exercises in rehabilitation programs.

GSH is known to be the most important antioxidant of the brain through removing ROS, renewing other antioxidant molecules, and repairing oxidized cell contents.[11] Low GSH levels are associated with decrease in growth and differentiation and increase in apoptosis in neuron cells.[11] It has been shown that GSH reduces oxidative stress-induced apoptosis.[28] In animal stroke models, it has shown that GSH administration provides a lower infarct area and better neurological outcomes.[29,30] In an another study, administration of 4 grams/day N-acetyl cysteine (NAC), which is a supplement containing thiol groups, for 2 days to patients who had a stroke in the last 24 hours provided a greater improvement in NIHSS scores in 3 months. [31] Similarly, it has been shown that 1-month changes in NIHSS scores of patients between 10-35 days post-stroke who received a cure (containing GSH, oxytocin, deproteinized calf serum and vitamins C, B1, B6, B12) were significant.[32] Changes in the GSH/GSSG ratio and GSSG levels play a controlling role on transcription factors such as NF-κB and AP-1, which play a role in the release of many cytokines and antioxidant molecules.[33] In our study, it was observed that high GSH levels and low GSSG levels were associated with better clinical outcomes. Also, the association between GSH and GSSG/GSH ratios and NIHSS and BI scores supports the idea that higher glutathione levels may be associated with the better clinical course of stroke.

A limitation in this study is that the oxidized-reduced thioredoxin system, which is another thiol-related balance, and the GPx, GR, thioredoxin reductase activities related to the thiol redox systems were not studied. Another limitation of this study is that this study was not an intervention study, therefore the effect of thiol-containing supplements (N-acetyl cysteine, glutathione, etc.) was not evaluated. In further studies, the effects of different rehabilitation programs such as conventional rehabilitation program and robot-assisted therapies on thiol redox systems can be evaluated together with activity measurements at the cortical level, which can be more objective in determining clinical data.

In conclusion, the better outcomes of patients with higher baseline antioxidant thiol groups suggest that active thiol groups may play a role in achieving better outcomes in subacute stroke. These findings suggest that correcting the deterioration in these balances may provide better outcomes. In addition, rehabilitation treatment increased clinical scores and additionally shifted intracellular and extracellular thiol groups to the antioxidant side. The relationship of better outcome scores with GSSG/GSH and SH/SS balances' parameters suggest that these parameters may be used as potential prognostic markers.

DISCLOSURE

Ethics: The study was approved by the Clinical Research Ethics Committee of to Bolu Abant Izzet Baysal University with approval number 2020/68. All subjects agreed to participate and signed the informed consent form. Declarations of interest: None

Financial support: None.

Conflict of interest: None

REFERENCES

1. Johnson CO, Nguyen M, Roth GA, *et al.* Global, regional, and national burden of stroke, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Neurol* 2019;18(5):439-58. doi: 10.1016/S1474-4422(19)30034-1.

2. Zheng M, Wang X, Yang J, Ma S, Wei Y, Liu S. Changes of complement and oxidative stress parameters in patients with acute cerebral infarction or cerebral hemorrhage and the clinical significance. *Exp Ther Med* 2020;19(1):703-9. doi: 10.3892/etm.2019.8229.
3. Kim J, Thayabaranathan T, Donnan GA, *et al*. Global stroke statistics 2019. *Int J Stroke* 2020;15(8):819-38. doi: 10.1177/1747493020909545.
4. Cenini G, Lloret A, Cascella R. Oxidative stress in neurodegenerative diseases: From a mitochondrial point of view. *Oxid Med Cell Longev* 2019;2019:2105607. doi: 10.1155/2019/2105607.
5. Aronowski J, Zhao X. Molecular pathophysiology of cerebral hemorrhage: secondary brain injury. *Stroke* 2011;42(6):1781-6. doi: 10.1161/STROKEAHA.110.596718.
6. Sies H. Oxidative stress: a concept in redox biology and medicine. *Redox Biol* 2015;4:180-3. doi: 10.1016/j.redox.2015.01.002.
7. Erel O, Neselioglu S. A novel and automated assay for thiol/disulphide homeostasis. *Clin Biochem* 2014;47(18):326-32. doi: 10.1016/j.clinbiochem.2014.09.026.
8. Alisik M, Alisik T, Nacir B, *et al.* Erythrocyte reduced/oxidized glutathione and serum thiol/disulfide homeostasis in patients with rheumatoid arthritis. *Clin Biochem* 2021;94:56-61. doi: 10.1016/j.clinbiochem.2021.04.023.
9. Doger C, Ozcan ATD, Erkilic E, *et al*. Oxidative stress in controlled hypotension: Assessment with a novel oxidative stress marker. *Clin Experimental Health Sci* 2021;11(2):324-9. doi: 10.33808/clinexphealthsci.798527.
10. Tsugawa S, Noda Y, Tarumi R, *et al.* Glutathione levels and activities of glutathione metabolism enzymes in patients with schizophrenia: A systematic review and meta-analysis. *J Psychopharmacol* 2019;33(10):1199-214. doi: 10.1177/0269881119845820.
11. Gerreth P, Maciejczyk M, Zalewska A, Gerreth K, Hojan K. Comprehensive evaluation of the oral health status, salivary gland function, and oxidative stress in the saliva of patients with subacute phase of stroke: A case-control study. *J Clin Med* 2020;9(7):2252. doi: 10.3390/jcm9072252.
12. Yilmaz AB, Gokhan S, Sener A, Erel O. Analysis of neutrophil/lymphocyte ratio and thiol/disulfide homeostasis parameters in patients admitted to the emergency department with ischemic stroke. *Pak J Med Sci* 2018;34(6):1418-23. doi: 10.12669/pjms.346.16242.
13. Bektas H, Vural G, Gumusyayla S, Deniz O, Alisik M, Erel O. Dynamic thiol-disulfide homeostasis in acute ischemic stroke patients. *Acta Neurol Belg* 2016;116(4):489-94. doi: 10.1007/s13760-016-0598-1.
14. Oliveira PVS, Laurindo FRM. Implications of plasma thiol redox in disease. *Clin Sci* (Lond). 2018;132(12):1257-80. doi: 10.1042/CS20180157.
15. Giustarini D, Santucci A, Bartolini D, Galli F, Rossi R. The age-dependent decline of the extracellular thiol-disulfide balance and its role in SARS-CoV-2 infection. *Redox Biol* 2021;41:101902. doi: 10.1016/j.redox.2021.101902.
16. Alisik M, Neselioglu S, Erel O. A colorimetric method to measure oxidized, reduced and total glutathione levels in erythrocytes. *J Laboratory Med* 2019;43(5):269-77. doi: 10.1515/labmed-2019-0098.
17. Kasner SE. Clinical interpretation and use of stroke scales. *Lancet Neurol* 2006;5(7):603-12. doi: 10.1016/S1474-4422(06)70495-1.
18. Kucukdeveci AA, Yavuzer G, Tennant A, Suldur N, Sonel B, Arasil T. Adaptation of the modified Barthel Index for use in physical medicine and rehabilitation in Turkey. *Scand J Rehabil Med* 2000;32(2):87-92.
19. Manolescu BN, Berteanu M, Oprea E, *et al*. Dynamic of oxidative and nitrosative stress markers during the convalescent period of stroke patients undergoing rehabilitation. *Ann Clin Biochem* 2011;48(Pt 4):338-43. doi: 10.1258/acb.2011.010243.
20. Tsai NW, Chang YT, Huang CR, *et al*. Association between oxidative stress and outcome in different subtypes of acute ischemic stroke. *Biomed Res Int* 2014;2014:256879. doi: 10.1155/2014/256879.
21. Leinonen JS, Ahonen JP, Lonnrot K, *et al*. Low plasma antioxidant activity is associated with high lesion volume and neurological impairment in stroke. *Stroke* 2000;31(1):33-9. doi: 10.1161/01.str.31.1.33.
22. Musumeci M, Sotgiu S, Persichilli S, *et al*. Role of SH levels and markers of immune response in the stroke. *Dis Markers* 2013;35(3):141-7. doi: 10.1155/2013/246205.
23. Kihoin N, Tanaka K, Okuno M, Okamoto T, Saura R. Exercise attenuates oxidative stress in patients with stroke. *Neurol Asia* 2016;21(1):7-16.
24. Friedenreich CM, Pialoux V, Wang Q, *et al*. Effects of exercise on markers of oxidative stress: an ancillary analysis of the Alberta Physical Activity and Breast Cancer Prevention Trial. *BMJ Open Sport Exerc Med* 2016;2(1):e000171. doi: 10.1136/bmjsem-2016-000171.
25. Ciancarelli I, Pistarini C, Carolei A, Tozzi Ciancarelli M. Oxidative stress in post-acute ischemic stroke patients: relevance of early intensive neurorehabilitation. *J Neurol Neurophysiol* 2013;4(154):10-20. doi: 10.2174/156720212803530717.
26. Hsieh YW, Lin KC, Korivi M, Lee TH, Wu CY, Wu KY. The reliability and predictive ability of a biomarker of oxidative DNA damage on functional outcomes after stroke rehabilitation. *Int J Mol Sci* 2014;15(4):6504-16. doi: 10.3390/ijms15046504.
27. Gomez-Cabrera MC, Salvador-Pascual A, Cabo H, Ferrando B, Vina J. Redox modulation of mitochondriogenesis in exercise. Does antioxidant supplementation blunt the benefits of exercise training? *Free Radic Biol Med* 2015;86:37-46. doi: 10.1016/j.freeradbiomed.2015.04.006.
28. Paul BD, Sbodio JI, Snyder SH. Cysteine metabolism in neuronal redox homeostasis. *Trends Pharmacol Sci* 2018;39(5):513-24. doi: 10.1016/j.tips.2018.02.007.
29. Kahl A, Stepanova A, Konrad C, *et al*. Critical role of flavin and glutathione in complex I-mediated bioenergetic failure in brain ischemia/reperfusion injury. *Stroke* 2018;49(5):1223-31. doi: 10.1161/STROKEAHA.117.019687.
30. Chen C, Ding QQ, Shen BY, *et al*. Insights into the authentic active ingredients and action sites

of oral exogenous glutathione in the treatment of ischemic brain injury based on pharmacokinetic-pharmacodynamic studies. *Drug Metab Dispos* 2020;48(1):52-62. doi: 10.1124/dmd.119.089458.
31. Sabetghadam M, Mazdeh M, Abolfathi P, Mohammadi Y, Mehrpooya M. Evidence for a beneficial effect of oral N-acetylcysteine on functional outcomes and inflammatory biomarkers in patients with acute ischemic stroke. *Neuropsychiatr Dis Treat* 2020;16:1265-78. doi: 10.2147/NDT.S241497.
32. Stancioiu F, Makk R. Post-stroke recovery of motor function with a new combination of medicines-A pilot study. *Eurasian J Med Oncol* 2019;3(3):167-81. doi: 10.14744/ejmo.2019.49036.
33. Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O. Oxidative stress and antioxidant defense. *World Allergy Organ J* 2012;5(1):9-19. doi: 10.1097/WOX.0b013e3182439613.