ORIGINAL ARTICLE

Evaluation of the thiol disulfide homeostasis in patients with traumatic hemorrhagic shock

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ABSTRACT

BACKGROUND: Traumatic hemorrhagic shock is a condition associated with a high mortality rate in the absence of timely diagnose and intervention. The class and severity of hemorrhagic shock are the key factors that guide the decisions in the management of these patients. This study aims to provide guidance for the timely administration of an appropriate treatment to patients with traumatic hemorrhagic shock, and thus, decrease morbidity and mortality, by determining shock severity and class more clearly with the use of the thiol disulfide homeostasis balance, which is an objective criterion.

METHODS: This controlled, prospective, and clinical study was conducted in the Emergency Medicine Clinic at the University of Health Sciences, Ankara Numune Training and Research Hospital between October 1, 2018 and April 30, 2019. Thiol disulfide homeostasis was assessed in blood collected from patients and healthy volunteers. A total of one hundred two patients were included; of whom 52 were female and male volunteer patients aged 18 or older who presented to the emergency department with traumatic hemorrhagic bleeding and fifty were control subjects.

RESULTS: Patient and control groups demonstrated significantly different native thiol, total thiol, disulfide, disulfide/native thiol, and disulfide/total thiol levels (P-values for native thiol, total thiol, disulfide, disulfide/native thiol, and disulfide/total thiol: 0.001>, 0.001>, 0.018, 0.002, and 0.002, respectively). According to pairwise comparisons; Class-3 hemorrhagic shock was associated with significantly lower native thiol and total thiol levels compared to Class-1 and Class-2 hemorrhagic shock (Comparison of Class-1 and Class-3 hemorrhagic shock: p-value for native thiol = 0.001, p-value for total thiol = 0.002) (Comparison of Class-2 and Class-3 hemorrhagic shock: p-value for native thiol = 0.009, p-value for total thiol = 0.006). Total thiol levels were found to be lower in patients who died compared to those who survived (p=0.040).

CONCLUSION: Thiol disulfide homeostasis data were found to be correlated with the shock class and mortality. The assessment of thiol disulfide homeostasis can serve as a guide in the determination of the severity and classification of the disease, evaluation of the prognosis, and management of the treatment in traumatic hemorrhagic shock patients.

Keywords: Disulfide; native thiol; shock stage; total thiol; traumatic hemorrhagic shock.

INTRODUCTION

According to data from the World Health Organization, trauma results in the loss of nine lives every minute and 5.8 million lives every year.[1] Trauma-related mortality is higher during 3 time periods. These time periods are: the first few minutes, the first few hours, and finally, the following days and weeks.[1] The first stage refers to death at the scene within minutes of the injury.[1] The first few hours; also known as the "Golden Hours," constitute a time period during which survival can be achieved by timely and effective intervention.[1] The third stage is the period during which mortality is encountered at later phases due to complications.[1]

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Patients who arrive in the emergency department during the "Golden Hours" have critical urgency, and immediate and effective intervention reduces mortality and morbidity. The most common type of shock encountered in cases of trauma is hemorrhagic shock.[1] Some parameters that can be assessed to determine the presence and severity of shock include; the shock index, modified shock index, base deficit, and lactate.[1–3] The most recent edition of the Advanced Trauma Life Support (ATLS) manual, which was published in 2018, included base deficit, a more concrete parameter, in the classification of hemorrhagic shock besides vital signs and emphasized the importance of blood lactate levels.[1] The ATLS classification of shock and the vital signs associated with each class are summarized in Table 1.

In a patient with traumatic hemorrhagic shock, the disruption of the balance between the pro-oxidant and anti-oxidant systems results in oxidative stress. Various cellular and tissue structures are damaged. Thiol molecules constitute an important component of the anti-oxidant system, which has a protective role against oxidative stress. Thiols are organic compounds that possess a sulfhydryl (-SH) group and can react with free radicals to counteract the harmful effects of the elevated levels of reactive oxygen species.[4,5] Thiol groups are reversed by being oxidized by oxidant molecules in the milieu. The consequent disulfide bond structures can be re-reduced to thiol groups and the thiol disulfide homeostasis balance is thus maintained.[6] The bidirectional thiol disulfide homeostasis balance, which could be measured unidirectionally since 1979, can now be assessed individually and collectively with a novel method developed by Erel and Neselioglu that can measure the levels of the two variables separately and in total.[6]

This study aims to contribute to the elucidation of the classification of hemorrhagic shock in traumatic hemorrhagic shock patients using the thiol disulfide homeostasis balance as an objective criterion to make a diagnosis in the early period, ensure that patients receive an appropriate treatment in a timely manner, and reduce morbidity and mortality. The use of such a laboratory indicator will improve the clinical outcomes by allowing scientific, timely, and appropriate patient management.

MATERIALS AND METHODS

This study was approved by the University of Health Sciences, Ankara Numune Training and Research Hospital Ethics Committee (Approval number: E-18-2166, date: 20.09.2018). After the relevant ethics approval was granted, a controlled, prospective, clinical study was conducted in the Emergency Medical Clinic at the University of Health Sciences, Yıldırım Beyazıt University Ankara Atatürk Training and Research Hospital between October 1, 2018, and April 30, 2019.

This study assessed the thiol disulfide balance in blood samples collected from volunteer patients and healthy individuals. A total of one hundred two patients were included; of whom 52 were female and male volunteer patients aged 18 or older who presented to our Emergency Medicine Clinic with traumatic hemorrhagic bleeding and fifty were control subjects recruited from healthy volunteer patient attendants. In the diagnosis of traumatic hemorrhagic shock, the following criteria specified in the classification chart in the 2018 ATLS manual were considered: approximate blood loss, heart rate, blood pressure, pulse pressure, urine output, respiratory rate, Glasgow Coma Scale score, base deficit, and need for blood products. Exclusion criteria were as follows: Lack of consent to participation, presence of diseases and conditions that could influence thiol disulfide homeostasis; pregnancy, smoking and/or alcohol intake, diabetes mellitus, cardiovascular diseases, cerebrovascular diseases, chronic kidney disease, rheumatoid arthritis, chronic liver disease, acute-chronic infections, malignancies, antioxidant medication use, Parkinson's disease, Alzheimer's disease; age <18 years; and isolated intracranial bleeding.

Table 1. Symptoms and findings by the amount of blood loss

Parameter	Class 1	Class 2	Class 3	Class 4
Blood loss (% blood volume)	<15%	15–30%	31–40%	>40%
Pulse rate	↔	↔/↑	↑	↑/↑↑
Blood pressure	↔	↔	↔/↓	↓
Pulse pressure	↔	↓	↓	↓
Respiratory rate	↔	↔	↔/↑	↑
Urine output	↔	↔	↓	↓↓
Glasgow Coma Scale	↔	↔	↓	↓
Base deficit	0- –2 mEq/L	–2- –6 mEq/L	–6- –10 mEq/L	–10 mEq/L or less
Need for blood products	Monitor	Possible	Yes	Massive transfusion protocol

Mutschler M, Nienaber U, Brockamp T, Wafaisade A, Wyen H, Peiniger S, et al. A critical reappraisal of the ATLS classification of hypovolemic shock: does it really reflect clinical reality? Resuscitation 2013;84(3):309–13. 10.1016/j.resuscitation.2012.07.012.

An informed consent form was obtained for the study from either the patient or their first-degree relatives. For each patient; demographic data at presentation, vital signs (blood pressure, pulse, body temperature, oxygen saturation, and respiratory rate), complete blood count, biochemical profile, blood gas, lactate, base deficit, shock index, modified shock index, type of injury, site of injury, urine output in the emergency department on the day of admission, and administered treatments were recorded in a patient study form.

Laboratory Analysis

To evaluate the thiol disulfide homeostasis balance described in the study; 10 µL blood was collected from the peripheral venous line into a biochemistry tube as soon as the patient was determined to meet the inclusion criteria and to have traumatic hemorrhagic shock according to the 2018 ATLS manual. Collected blood samples were centrifuged at 3600 rpm for 10 min and stored at −80°C in the biochemistry laboratory of Ankara Numune Training and Research Hospital. After the collection of all samples, all were thawed simultaneously, and blood thiol disulfide parameters were assessed according to the novel automatic spectrophotometric measurement method developed by Erel and Neselioglu, [6] using a Roche Hitachi Cobas c501 automatic analyzer, in the biochemistry laboratory of Yıldırım Beyazıt University, Ankara Atatürk Training and Research Hospital. According to the principles of the test; disulfide bonds were reduced using sodium borohydride to produce free functional thiol groups. Formaldehyde was used to remove unused sodium borohydride and DTNB (5,5'-dithiobis-2- nitrobenzoic acid) products. Following this procedure, both reduced and native thiol groups were identified. The amount of dynamic disulfide bonds was determined by computing the half of the difference of total thiol and native thiol groups. After determining the amounts of native thiol, total thiol, and disulfide; disulfide/total thiol, native thiol/total thiol, and disulfide/native thiol percent ratios were calculated.

Statistical Analysis

Statistical analyses were conducted using SPSS 23.0 for Windows. Descriptive statistics are shown as frequency and percentages for categorical variables and as mean, standard deviation, minimum, maximum, and median values for quantitative variables. For the comparison of quantitative variables in independent groups, normality was checked using the Kolmogorov–Smirnov test. Since at least one group did not conform to a normal distribution on each criterion, pairwise group comparisons used the Mann–Whitney U-test and multiple groups were compared using the Kruskal–Wallis test. When significant differences were determined by the Kruskal–Wallis test, subgroups were subjected to post hoc pairwise analysis using the Mann–Whitney U-test. Chi-square analysis was used in the comparison of ratios between independent groups. Correlations between quantitative variables were computed using Spearman Rho Correlation analysis. Statistical significance was taken as $p<0.05$.

RESULTS

Fifty-two patients were included in this study, of whom nine (17%) were female and 43 (82.7%) were male. The control group consisted of twenty (40%) females and thirty (60%) males. Mean and median age were determined as 43.6±15.7 and 42.5 (18–80) for the patient group and as 33.1±10.3 and 31 (18–57) for the control group, respectively (Fig. 1). Median values were used in the statistical analysis of age and gender data.

Presenting complaints of the patient group were, in descending order of frequency, as follows; sharp instrument injury (26.9%), motor vehicle accident (25%), pedestrian injury (23.1%), fall from height (17.3%), motorcycle accident (1.9%), gunshot wound (1.9%), and physical assault (1.9%). The most common sites of trauma were, in descending order of frequency, as follows; thorax (67.3%), abdomen (42.3%), head-neck (40.4%), extremities (36.5%), and pelvis (23.1%).

Shock classes determined at the initial admission were, in descending order of frequency, as follows; second-class shock (34.6%), third-class shock (28.8%), first-class shock (26.9%), and fourth-class shock (9.6%) (Table 2).

Median values of the patients' vital signs were as follows; heart rate, 126 (72–165) bpm; systolic blood pressure, 115 (18–186) mmHg; diastolic blood pressure, 65 (25–100) mmHg; pulse pressure, 46.5 (20–96) mmHg; respiratory rate, and 23.5 (15–42) bpm; oxygen saturation, 92% (70–98) (Table 2). In the patient group; mean urine output was 22.7 ml/hour, mean shock index was 1.24, and mean modified shock index was 1.76 (Table 2). There were 31 (75%) patients with blood gas lactate levels of 2 mmol/L or higher (Table 2). Base deficit was between 0 and −2 mEg/L in 14 (26.9%) patients, between −2 and −6 mEq/L in 22 (42.3%) patients, between −6 and −10 mEq/L in 7 (13.5%) patients, and −10 or less in 9 (17.3%) patients (Table 2). Median white blood cell ($\times10^3$/µL), red blood cell ($\times10^6$/µL), hemoglobin (g/dL), and hematocrit (%) levels of the patient group were 15 (4.7–38.7), 4.61 (2.49–6.05), 13.55 (6.8–17.4), and 40.2 (20.1–51.6), respectively.

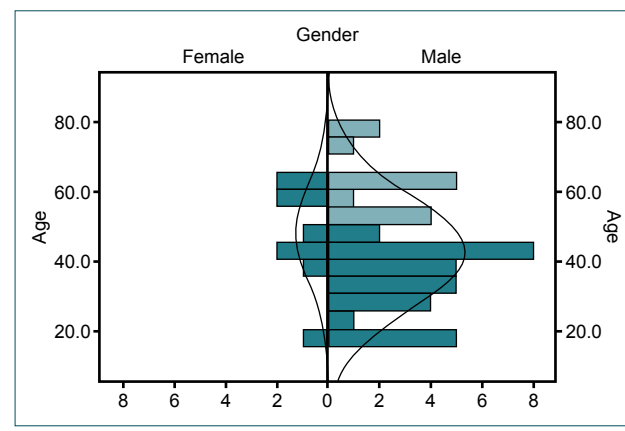

Figure 1. Age distribution of the patients by gender.

Table 2. Distribution of shock class, Glasgow Coma Scale score, vital signs, urine output, shock index, modified shock index, lactate, and base deficit in the patient group at admission to the emergency department

	Patient group
	Mean±SD/median (min-max)/n (%)
Shock class	
1	14 (26.9)
2	18 (34.6)
3	15 (28.8)
4	5 (9.6)
Glasgow Coma Scale	11.1±4.6/14 (3–15)
Vital signs	
Heart rate (bpm)	123±24.3/126 (72–165)
Systolic blood pressure (mmHg)	108±31.5/115 (18–186)
Diastolic blood pressure (mmHg)	62.8±17.7/65 (25–100)
Pulse pressure (mmHg)	47±14/46.5 (20–96)
Respiratory rate (bpm)	23.9±6.6/23.5 (15–42)
Oxygen saturation (%)	91.2±6.5/92 (70–98)
Urine output (mL/h)	22.7±11.8/25 (0–40)
Shock index	1.24±0.56/1.03 (0.61–2.72)
Modified shock index	1.76±0.82/1.43 (0.82–3.7)
Lactate (mmol/L)	3.9±2.97/3 (0.8–16)
<2 mmol/L	13 (25%)
≥2 mmol/L	39 (75%)
Base deficit (mEq/L)	-4.43±6.04/-2.6 (-21.4–3.1)
Between 0 and -2 mEq/L	14 (26.9)
Between -2 and -6 mEq/L	22 (42.3)
Between -6 and -10 mEq/L	7 (13.5)
-10 or less	9 (17.3)

SD: Standard deviation; Min: Minimum; Max: Maximum.

Table 3. Distribution of fluids and blood products administered to the patients

	Patient group	
	n	%
Crystalloid/blood replacement		
Only monitoring	7	13.4
Only crystalloid	20	38.5
Crystalloid, blood and blood products	25	48.1
Erythrocyte suspension (units)		
1	11	44
2	10	40
3	3	12
4	1	4

Table 4. Outcome in the emergency department and mortality outcome at the end of the study

	Patient group	
	n	%
Outcome at the emergency department on the day of admission		
Discharged on an outpatient basis	4	7.7
Admitted to a department	14	26.9
Admitted to the intensive care unit	17	32.7
Emergency surgery	14	26.9
Died at the emergency department	3	5.8
Mortality outcome at the end of the study		
Alive	44	84.6
Dead	8	15.4

Seven patients (13.4%) were monitored without any fluid or blood product transfusion, twenty (38.5%) received only crystalloid infusion, and 25 (48.1%) received crystalloid, blood and blood product replacement (Table 3). The average volume of crystalloid infusion was approximately one liter. Of the 25 patients who were transfused with erythrocyte suspension; 11 (44%) received one unit, ten (40%) received two units, three (12%) received three units, and one (4%) received four units of erythrocyte suspension replacement (Table 3). Where possible, primarily, and bleeding were controlled, and balanced fluid resuscitation was initiated. According to the restrictive fluid replacement guidelines of the ATLS 10 manual; blood product replacement was initiated in the absence of response to crystalloid resuscitation of 1 L. Blood product replacement was performed according to the guidelines of the ATLS 10 manual, depending on the rapid response, transient response, minimal response, and no response states of the patients. Again, in accordance with the guidelines of the ATLS 10 manual; 0 Rh-blood products were obtained for the patients to ensure a rapid blood product provision process, as a full cross-matching procedure would last longer than an hour. Therefore, blood product replacement was initiated within the 1st h in the emergency department, where required.

Four (7.7%) patients were discharged on an outpatient basis, 14 (26.9%) were admitted to a department, 17 (32.7%) were admitted to the intensive care unit, 14 (26.9%) underwent emergency operations, and three (5.8%) died in the emergency department (Table 4). In this study, patients were followed-up for 3 months, and a total of eight patients died during this period. Of these eight patients; three died in the

emergency department and two during an emergency operation, within the first 24 h. The remaining three patients died while admitted to the intensive care unit. Of these three patients who died in the intensive care unit; one died on the 6th day after admission due to sepsis, one died on the 12th day after admission due to multiple organ failure, and one died on the 18th day after admission due to multiple organ failure.

The patient group showed lower native thiol, total thiol, and disulfide levels compared with the control group (Table 5). Disulfide/native thiol and disulfide/total thiol ratios were significantly higher in the patient group compared with the control group (Table 5). Median values were used in the relevant statistical analyses.

Native thiol and total thiol showed a moderate positive correlation with the Glasgow Coma Scale score, urine output, diastolic blood pressure, oxygen saturation, base deficit, red blood cell, hemoglobin and hematocrit levels; and a moderate negative correlation with respiratory rate, heart rate, shock index, modified shock index, and lactate. In summary; native thiol and total thiol decrease as Glasgow Coma Scale score, diastolic blood pressure, urine output, oxygen saturation, base deficit, red blood cell, hemoglobin, and hematocrit values decrease; and as heart rate, respiratory rate, shock index, modified shock index, and lactate values increase. Disulfide levels decrease as heart rate and respiratory rate increase and as diastolic blood pressure decreases. Total thiol shows a weak positive correlation with systolic blood pressure. The correlations of the mentioned parameters with thiol disulfide homeostasis are presented in Table 6.

The change in thiol disulfide homeostasis balance with respect to shock class is presented in Table 7. Inspecting all of these groups reveals that native thiol and total thiol levels vary depending on the shock class. Median values were used in the relevant statistical analyses.

The groups that these shock class-associated differences in native thiol and total thiol levels originated from were investigated by pairwise comparisons. The comparison of Classes 1 and 3 determined P-values of 0.001, 0.002, and 0.022 for native thiol, total thiol, and disulfide, respectively; whereas the comparison of Classes 2 and 3 determined P-values of 0.009 and 0.006 for native thiol and total thiol, respectively. The distributions of native thiol and total thiol by shock class are shown in Figures 2 and 3.

Pairwise comparisons of patients who were discharged from the ED on an outpatient basis versus others, patients admitted to a department versus patients admitted to the intensive care unit, patients who died in the ED versus others, patients who died during the study versus patients who survived revealed that total thiol levels were lower in patients who died during the study compared with those who survived (p=0.040) (Table 8). Other pairwise group comparisons did not produce statistically significant results.

DISCUSSION

This prospective, controlled clinical study determined statistically significant differences between the patient and the control groups in all parameters concerning the thiol disulfide ho-

Table 5. Thiol disulphide homeostasis balance in the patient and control groups

	Patient group		p*
	Patient	Control	
	Mean±SD/median (min-max)	Mean±SD/median (min-max)	
Thiol disulfide homeostasis			
Native Thiol (µmol/L)	337.2±86.9	427.5±46.1	**<0.001**
	344.1 (140.5–508.9)	423.4(352.2–534.6)	
Total Thiol (µmol/L)	376.3±92.3	470.2±48.7	**<0.001**
	385 (166.3–542.8)	473.5 (383.4–588.5)	
Disulfide (µmol/L)	19.5±4.8	21.4±5.1	**0.018**
	19.2 (9.2–33.4)	21.1 (4.6–30.5)	
Disulfide/Native Thiol (x100)	6±1.5	5±1.2	**0.002**
	5.8 (2.5–9.4)	5.2 (1.1–7.5)	
Disulfide/Total Thiol (x100)	5.3±1.2	4.6±1	**0.002**
	5.2 (2.4–7.9)	4.7 (1.1–6.5)	
Native/Total Thiol (x100)	89.3±2.3	90.9±2.1	**0.002**
	89.6 (84.2–95.3)	90.6 (87–97.9)	

*Mann-Whitney U test. SD: Standard deviation; Min: Minimum; Max: Maximum. Bold font is used for statistically significant correlations.

Table 6. Correlation of the thiol disulfide homeostasis balance with the patients' Glasgow Coma Scale scores, vital signs, and certain blood parameters

		Native thiol (µmol/L)	Total thiol (µmol/L)	Disulphide (µmol/L)
Glasgow Coma Scale score	r	.335*	.329*	0.191
	p	0.015	0.017	0.175
Urine output (mL/h)	r	.470**	.479**	.288*
	p	<0.001	<0.001	0.038
Diastolic blood pressure (mmHg)	r	.444**	.441**	.279*
	p	0.001	0.001	0.045
Oxygen saturation (%)	r	.369**	.369**	0.220
	p	0.007	0.007	0.117
Base deficit (mEq/L)	r	.484**	.487**	.321*
	p	<0.001	<0.001	0.020
Red blood cells (x10^6/µL)	r	.447**	.451**	.311*
	p	0.001	0.001	0.025
Hemoglobin (g/dL)	r	.562**	.570**	.374**
	p	<0.001	<0.001	0.006
Hematocrit (%)	r	.541**	.544**	.326*
	p	<0.001	<0.001	0.018
Respiratory rate (bpm)	r	-.501**	-.515**	-.291*
	p	<0.001	<0.001	0.037
Heart rate (bpm)	r	-.366**	-.376**	-.306*
	p	0.008	0.006	0.027
Shock index	r	-.375**	-.385**	-.310*
	p	0.006	0.005	0.026
Modified shock index	r	-.430**	-.438**	-.335*
	p	0.001	0.001	0.015
Lactate (mmol/L)	r	-.395**	-.403**	-.324*
	p	0.004	0.003	0.019
Systolic blood pressure (mmHg)	r	0.272	.274*	0.193
	p	0.051	0.050	0.171

Spearman Rho Correlation. *Indicates r values when p<0.05. **Indicates r values when p<0.01. Bold font is used for statistically significant correlations.

meostasis balance that were tested. Pairwise comparisons of shock class within the patient group revealed lower native thiol and total thiol levels in the Class-3 hemorrhagic shock class compared with Class-1 and Class-2 hemorrhagic shock classes.

It is a well-established fact that free oxygen radicals induce oxidative stress in trauma patients. These oxygen radicals can inflict reversible or irreversible damage to biomolecules. There is strong evidence suggesting that free oxygen radicals play a key role in the damage resulting from acute trauma and initiate tissue damage by causing lipid peroxidation.[7] In a study conducted in 1993, Hall et al.[8] measured levels of the OH-radical in rats with acute experimental head injury with the spectrophotometric method and showed that OH-radicals started increasing immediately after trauma and attained a significant peak level at 1 h after trauma. Spinal cord injuries are also associated with free radical production and lipid peroxidation in the early period.[8] In a study performed on rats; lipid peroxidation was found to be at a maximum level 1 h after spinal cord injury.[9]

Thiol is one of the main antioxidant molecules that scavenge free oxygen radicals, and thiol molecules are found in either free or oxidized form in the plasma.[4,10] The plasma thiol pool is comprised predominantly of albumin and protein thiols, and to a lesser extent, of low molecular weight compounds such as glutathione, homocysteine, cysteine, and γ glutamine. When oxidative stress is elevated, thiol levels are reduced in order to neutralize free oxygen radicals, and the -SH groups of thiols play a key role in this process. In the presence of oxidative

Table 7. Differences in thiol disulfide homeostasis balance by shock class

	Shock class				p
	1	2	3	4	
	Mean±SD/median (min-max)	Mean±SD/median (min-max)	Mean±SD/median (min-max)	Mean±SD/median (min-max)	
Native thiol (µmol/L)	380.1±71.8 / 386.8 (284.5–495.6)	359.5±84.6 / 362.8 (220.1–508.9)	276.2±76.5 / 265.6 (140.5–399.9)	320.4±75.3 / 335.7 (230–400.9)	**0.008**
Total thiol (µmol/L)	423.6±789 / 430.2 (321.9–542.8)	399.4±87.9 / 409.5 (242.5–534.2)	310.2±81.9 / 306.7 (166.3–441.3)	358.7±71.3 / 376.1 (273.3–436.3)	**0.007**
Disulphide (µmol/L)	21.7±5.6 / 20.1 (15.8–33.4)	20±4.6 / 20.2 (11.2–26.7)	17±3.9 / 17.4 (9.2–22.5)	19.2±2.3 / 20.2 (15.9–21.6)	0.108
Disulphide/Native thiol (x100)	5.7±1.1 / 5.4 (4.4–7.8)	5.7±1.5 / 5.5 (2.5–9.1)	6.4±1.5 / 6.2 (3.5–9.1)	6.4±2.3 / 6 (4.2–9.4)	0.615
Disulphide/Total thiol (x100)	5.1±0.9 / 4.9 (4–6.8)	5.1±1.2 / 5 (2.4–7.7)	5.6±1.2 / 5.5 (3.3–7.7)	5.6±1.8 / 5.4 (3.9–7.9)	0.615
Native/Total thiol (x100)	89.7±1.8 / 90.2 (86.4–91.9)	89.7±2.3 / 90 (84.6–95.3)	88.7±2.4 / 88.9 (84.5–93.4)	88.8±3.5 / 89.3 (84.2–92.3)	0.615

*Kruskal Wallis test. SD: Standard deviation; Min: Minimum; Max: Maximum. Bold font is used for statistically significant values.

Table 8. Pairwise group comparisons of the thiol disulfide homeostasis balance based on patient outcomes during the study period

	p-values*			
	Discharged on an outpatient basis / others	Admitted to a department / admitted to the ICU	Died in the ED / others	Total dead / alive
Native thiol (µmol/L)	0.107	0.242	0.860	0.054
Total thiol (µmol/L)	0.092	0.275	0.768	**0.040**
Disulfide (µmol/L)	0.080	0.258	0.984	0.254

*Mann-Whitney U test. ICU: Intensive care unit; ED: Emergency department. Bold font is used for statistically significant correlations.

stress, reversible disulfide bonds are observed between protein thiols and low molecular weight compounds. These bonds can be reduced to thiols again to maintain the thiol disulfide homeostasis.[11,12] Thus, dynamic thiol disulfide homeostasis is

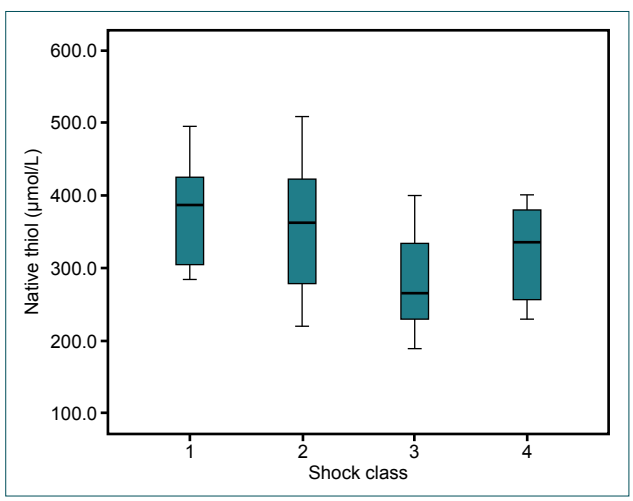

Figure 2. Distribution of native thiol levels by shock class.

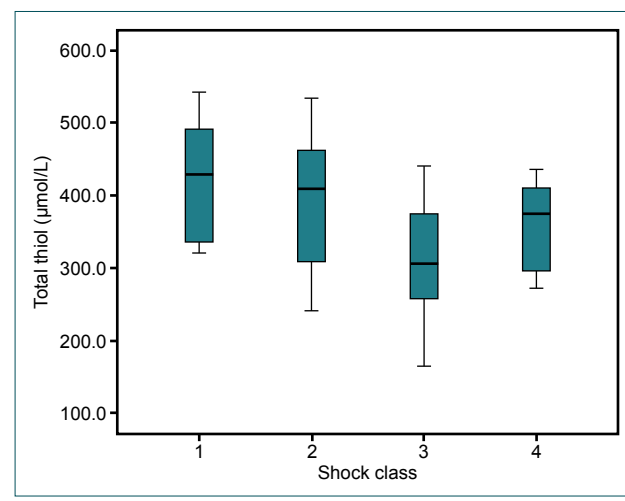

Figure 3. Distribution of total thiol levels by shock class

preserved. The state of dynamic thiol disulfide homeostasis plays a critical role in; antioxidant protection, detoxification, signal transduction, apoptosis, regulation of enzymatic activity, transcription factors, and cellular signaling mechanisms. Dynamic thiol disulfide homeostasis in plasma can be assessed using a novel test developed by Erel and Neselioglu, which is practical, automatic, and optionally manual spectrophotometric.[6] Thiol disulfide homeostasis, which is a novel parameter of oxidative stress, has been investigated in a variety of diseases, such as coronary heart disease, multiple sclerosis, hypertension, diabetes mellitus, Alzheimer's disease, and preeclampsia in the recent years.[4,5,13–18] Our literature review did not identify any studies that investigated thiol disulfide homeostasis in traumatic hemorrhagic shock patients, and the present study is noteworthy as the first study on this topic.

Coşkun et al.[19] assessed diabetic and non-diabetic patients with chronic renal failure and reported a statistically significant difference between the patient and control groups in terms of total thiol and free thiol levels. Total thiol and free thiol levels were found to be significantly lower in the patient group with renal failure compared with controls; and in parallel to this finding, these levels showed a further decline in diabetic patients with renal failure. Excess production of oxidative products is known to be involved in the etiology of Chronic Renal Failure and Diabetes Mellitus.[20,21] In congruence with the results of our study, these studies demonstrated a decrease in thiol levels in conditions associated with elevated oxidative stress.

In a study by Güllü-Haydar et al.[22] that evaluated thiol disulfide homeostasis in patients who presented to the emergency department with acute pancreatitis, patients with acute pancreatitis were shown to have lower thiol levels than the control group, with statistical significance. In agreement with the present study, the cited study determined lower thiol and native thiol levels and higher disulfide/native thiol and disulfide/total thiol ratios in the patient group compared with controls. In a study performed on patients symptomatically diagnosed with bladder calculi and planned to undergo laparoscopic cholecystectomy, Polat et al.[23] determined lower preoperative and postoperative Native Thiol, Total Thiol, and Disulfide levels in the group planned to undergo surgery than the healthy control group.

Ahmet and Avcı measured total antioxidant and oxidant levels and the oxidative stress index in patients with head trauma, and reported statistically significant differences in the levels of oxidative stress parameters, with lower levels of parameters indicating the antioxidant capacity.[24] In a study by Buyukaslan et al.[25] that included a total of one hundred twenty-eight participants, of whom 73 were patients and 55 were controls, and investigated the serum thiol disulfide homeostasis in patients who presented to the emergency department with gunshot wounds; the authors stated that thiol levels of the patients decreased in correlation with their Glasgow Coma Scale scores and that native thiol was an independent indicator of the Glasgow coma scale. Native thiol and total thiol levels also showed a moderate positive correlation with the Glasgow Coma Scale score in the present study; and in both studies, native thiol and total thiol levels decreased as the Glasgow Coma Scale score decreased. Consistent with our study, the patient group manifested lower native thiol, total thiol and disulfide levels and higher disulfide/native thiol and disulfide/total thiol ratios compared with the control group.

In a study that Erel and Neselioglu conducted to develop a novel and automatic test that would assess thiol disulfide homeostasis; plasma disulfide levels were shown to be higher in smokers and patients with diabetes, obesity, and pneumonia; and lower in patients with multiple myeloma, bladder cancer, and colon cancer.[6] While some studies determined lower disulfide levels in the patient group compared with the control group, in line with our results,[23,25] some determined higher levels.[22,26] As can be seen, patients with oxidative stress can show higher or lower disulfide levels than controls. As mentioned above; a normal, healthy functioning is maintained in the body while the oxidant and antioxidant systems are in balance, and oxidative stress occurs when the oxidant-antioxidant balance is disrupted in favor of oxidant activity. The results of our study can be better understood in this context. Considering that thiols oxidized by the oxidant molecules in the milieu transform into reversible disulfide bond (-S-S-) structures, and that the consequent disulfide bond structures can be re-reduced to thiol groups, and in short, that the thiol disulfide homeostasis balance is a dynamic process; and assessing disulfide and thiol levels concurrently, rather than disulfide as a single parameter, would allow us to view the process through a broader perspective, and reach a more accurate interpretation. The higher disulfide/thiol ratio determined in the patient group and its further increase in more advanced shock classes suggest that oxidant activity is dominant over antioxidant activity. In the present study, disulfide/total thiol and disulfide/native thiol ratios showed a statistically significant increase compared with the control group (p=0.002 and 0.002, respectively). In conclusion, this study shows that thiol disulfide homeostasis shifts in favor of disulfide production due to thiol oxidation.

The traumatic hemorrhagic shock classification system provides guidance to clinicians as to the severity of the disease, the gravity of the clinical findings, and the development of a treatment plan. The most recent ATLS 10 guideline also includes base deficit values in the classification of hemorrhagic shock. The fact that the classification was based on an objective laboratory parameter such as base deficit was deemed promising for the improvement of the classification by the inclusion of oxidative stress parameters such as thiol disulfide homeostasis balance. The results we obtained in this study appear to confirm this prediction. In our study, native thiol and total thiol levels showed a gradual decrease as the hemorrhagic shock class increased in patients with Class-1, Class-2, and Class-3 hemorrhagic shock. Meanwhile, although native thiol

and total thiol showed a slight increase in Class-4 hemorrhagic shock, they did not approach the levels seen in Class-1 hemorrhagic shock. Although thiol levels demonstrated a limited increase in Class-4 hemorrhagic shock, the evaluation of other parameters involved in thiol disulfide homeostasis clearly demonstrate that disulfide/total thiol and disulfide/native thiol ratios increase as the shock class increases, and in summary, that there is a shift in the thiol disulfide homeostasis in favor of disulfide (Table 7). Class-3 hemorrhagic shock was associated with lower native thiol and total thiol levels compared with Class-1 and Class-2 hemorrhagic shock (Comparison of Class-1 and Class-3 hemorrhagic shock; p-value for native thiol= 0.001, p-value for total thiol=0.002) (Comparison of Class-2 and Class-3 hemorrhagic shock; p-value for native thiol = 0.009, p-value for total thiol = 0.006). Shock index and the modified shock index, which are valuable indicators of trauma severity, had a negative statistical correlation with thiol disulfide homeostasis (shock index: p-value for native thiol = 0.006, p-value for total thiol = 0.005; modified shock index: p-value for native thiol = 0.001, p-value for total thiol = 0.001). The relation of mortality, which is another parameter that reflects the severity of trauma, with the assessed parameters becomes more important. In a study by Topuz et al.;[26] native thiol levels were lower and disulfide levels were higher in the acute pulmonary embolism group compared with the control group, and native thiol and disulfide levels were reported to be independent factors of mortality in acute pulmonary embolism patients. In the present study, total thiol levels were found to be lower in patients who died than those who survived. Accordingly, we reason that low thiol levels in patients who present with traumatic hemorrhagic shock may provide insight to the clinician as to a high risk of mortality. All of these results obtained in the present study confirm the notion that antioxidant activity dramatically decreases as the severity of traumatic hemorrhagic shock increases.

In this study; native thiol and total thiol showed a moderate positive correlation with urine output, base deficit, oxygen saturation, red blood cell, hemoglobin, and hematocrit levels; and a moderate negative correlation with lactate. We did not identify any studies in the literature that directly investigated the parameters listed above. However, the study conducted by Topuz et al.[26] that reports on the prognostic value of thiol disulfide homeostasis in patients with acute pulmonary thromboembolism presents data that can be interpreted similarly. When we inspect our study and the cited study more thoroughly.

In the study by Topuz et al.,[26] native thiol and total thiol levels were reported to show a positive correlation with blood pressure and a negative correlation with heart rate. Accordingly, our study shows that blood loss, hypotension, and an advanced shock class are associated with elevated oxidative stress, and hence, with a decrease in native thiol and total thiol levels.

In summary; all parameters associated with the thiol disulfide homeostasis showed statistically significant differences between the patient and control groups. There were also statistically significant differences within the patient group with respect to hemorrhagic shock classes. Total thiol levels were determined to be lower in patients who died than those who survived. According to these results, oxidative stress parameters are associated with shock class, and can be recognized as indicators to be considered in the determination of the severity and classification of the disease.

Limitations

A limited number of patients and control subjects could be included due to the single-center design of our study. Furthermore, patient and control groups were not matched in terms of age and gender. Multi-center studies can produce further results with the inclusion of more patients and controls. As these oxidative stress parameters have only recently become topics of investigation, existing studies to be used as a reference in the comparisons and discussions are limited. Future studies will generate more literature support on this subject.

Conclusion

According to these results; assessing thiol disulfide homeostasis can provide insight to the clinician regarding the classification and severity of the disease, and the prediction of the prognosis and mortality in traumatic hemorrhagic shock patients. Advancing the management of traumatic hemorrhagic shock patients with the use of objective and quantifiable data, as presented in this study, will improve the outcomes.

Ethics Committee Approval: This study was approved by the University of Health Sciences, Ankara Numune Training and Research Hospital Ethics Committee (Approval number: E-18-2166, date: 20.09.2018).

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REFERENCES

1. American College of Surgeons. Advanced Trauma Life Support. 10th ed. Chicago: American College of Surgeons; 2018.
2. Rady MY, Nightingale P, Little RA, Edwards JD. Shock index: A re-evaluation in acute circulatory failure. Resuscitation 1992;23:227–34. [CrossRef]
3. Liu YC, Liu JH, Fang ZA, Shan GL, Xu J, Qi ZW, et al. Modified shock index and mortality rate of emergency patients. World J Emerg Med 2012;3:114–7. [CrossRef]
4. Ates I, Ozkayar N, Inan B, Yilmaz FM, Topcuoglu C, Neselioglu S, et al. Dynamic thiol/disulphide homeostasis in patients with newly diagnosed primary hypertension. J Am Soc Hypertens 2016;10:159–66. [CrossRef]
5. Kundi H, Ates I, Kiziltunc E, Cetin M, Cicekcioglu H, Neselioglu S, et

al. A novel oxidative stress marker in acute myocardial infarction; thiol/disulphide homeostasis. Am J Emerg Med 2015;33:1567–71. [CrossRef]
6. Erel O, Neselioglu S. A novel and automated assay for thiol/disulphide homeostasis. Clin Biochem 2014;47:326–32. [CrossRef]
7. Cho S, Park EM, Febbraio M, Anrather J, Park L, Racchumi G, et al. The class B scavenger receptor CD36 mediates free radical production and tissue injury in cerebral ischemia. J Neurosci 2005;25:2504–12. [CrossRef]
8. Hall TR, Wallin R, Reinhart GD, Hutson SM. Branched chain aminotransferase isoenzymes. Purification and characterization of the rat brain isoenzyme. J Biol Chem 1993;268:3092–8. [CrossRef]
9. Barut S, Canbolat A, Bilge T, Aydin Y, Cokneseli B, Kaya U. Lipid peroxidation in experimental spinal cord injury: Time-level relationship. Neurosurg Rev 1993;16:53–9. [CrossRef]
10. Bedreag OH, Rogobete AF, Sarandan M, Cradigati AC, Papurica M, Dumbuleu MC, et al. Oxidative stress in severe pulmonary trauma in critical ill patients. Antioxidant therapy in patients with multiple trauma-a review. Anaesthesiol Intensive Ther 2015;47:351–9. [CrossRef]
11. Guly HR, Bouamra O, Little R, Dark P, Coats T, Driscoll P, et al. Testing the validity of the ATLS classification of hypovolaemic shock. Resuscitation 2010;81:1142–7. [CrossRef]
12. Nicks BA, Gaillard J. Approach to shock. In: Tintinalli JE, Stapczynski JS, Ma OJ, Yealy DM, Meckler GD, Cline DM, editors. Tintinalli's Emergency Medicine: A Comprohensive Study Guide. 8th ed. United States: McGraw-Hill Education; 2016. p. 63–9.
13. Ates I, Kaplan M, Yuksel M, Mese D, Alisik M, Erel O, et al. Determination of thiol/disulphide homeostasis in Type 1 diabetes mellitus and the factors associated with thiol oxidation. Endocrine 2016;51:47–51. [CrossRef]
14. Kundi H, Erel O, Balun A, Cicekcioglu H, Cetin M, Kiziltunc E, et al. Association of thiol/disulfide ratio with syntax score in patients with NSTEMI. Scand Cardiovasc J 2015;49:95–100. [CrossRef]
15. Altiparmak IH, Erkus ME, Sezen H, Demirbag R, Kaya Z, Sezen Y, et al. Evaluation of thiol levels, thiol/disulfide homeostasis and their relation with inflammation in cardiac syndrome X. Coron Artery Dis 2016;27:295–301.
16. Ozler S, Erel O, Oztas E, Ersoy AO, Ergin M, Sucak A, et al. Serum thiol/disulphide homeostasis in preeclampsia. Hypertens Pregnancy 2015;34:474–85. [CrossRef]
17. Gumusyayla S, Vural G, Bektas H, Deniz O, Neselioglu S, Erel O. A novel oxidative stress marker in patients with Alzheimer's disease: Dynamic thiol-disulphide homeostasis. Acta Neuropsychiatr 2016;28:315–20.
18. Guney T, Kanat IF, Alkan A, Alisik M, Akinci S, Silay K, et al. Assessment of serum thiol/disulfide homeostasis in multiple myeloma patients by a new method. Redox Rep 2017;22:246–51. [CrossRef]
19. Coşkun C, Emre HÖ, Gümüş A, Uzun S, Karadağ S, Behlül A. Diyabetik ve diyabetik olmayan kronik böbrek yetmezliğinde dinamik tiyol disülfit homeostazi ve ileri protein oksidasyon ürünleri (AOPPs). Deneysel Tıp Araştırma Enstitüsü Dergisi 2016;6:1–9.
20. Jan G. Oxidative stress in chronic renal failure. Nephrol Dial Transplant 2001;16:2135–7. [CrossRef]
21. Suziy MB, Lucas JS, Glaucevane SG, Luíza AR, Marília OF, Sandra ML. Oxidative stress as an underlying contributor in the development of chronic complications in diabetes mellitus. Int J Mol 2013;14:3265–84.
22. Güllü-Haydar F, Otal Y, Şener A, Günaydın GP, İçme F. The thiol-disulphide homeostasis in patients with acute pancreatitis and its relation with other blood parameters. Ulus Travma Acil Cerrahi Derg 2020;26:37–42.
23. Polat M, Ozcan O, Sahan L, Ustundag-Budak Y, Alisik M, Yilmaz N, et al. Changes in thiol-disulfide homeostasis of the body to surgical trauma in laparoscopic cholecystectomy patients. J Laparoendosc Adv Surg Tech A 2016;26:992–6. [CrossRef]
24. Çakır A. Kafa Travmalı Hastalarda Oksidatif Stres ve Antioksidan Kapasite Ölçümü. Turkey: Harran Üniversitesi, Uzmanlık Tezi; 2009. p. 37–8.
25. Buyukaslan H, Gulacti U, Gokdemir MT, Giden R, Celik H, Erel O et al. Serum thiol levels and thiol/disulphide homeostasis in gunshot injuries. Eur J Trauma Emerg Surg 2019;45:167–74. [CrossRef]
26. Topuz M, Kaplan M, Akkus O, Sen O, Yunsel HD, Allahverdiyev S, et al. The prognostic importance of thiol/disulfide homeostasis in patients with acute pulmonary thromboembolism. Am J Emerg Med 2016;34:2315–9.

ORİJİNAL ÇALIŞMA - *ÖZET*

Travmatik hemorajik şokta olan hastalarda tiyol disülfid homeostazisinin değerlendirilmesi

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AMAÇ: Travmatik hemorajik şok, hızlıca tanınıp müdahale edilmediği takdirde yüksek mortalite ile seyreden bir durumdur. Hemorajik şokun evresi ve şiddeti bu hastaların yönetiminde kararları belirleyen temel faktördür. Bu çalışmada, travmatik hemorajik şokta olan hastalarda objektif bir kriter olan tiyol disülfid homeostazisi dengesi ile şokun şiddet ve derecesini daha net ortaya koyarak, hızlı bir şekilde hastaların uygun tedaviyi alabilmesine rehberlik etmek ve böylece morbidite ve mortaliteyi azaltmak amaçlandı.
GEREÇ VE YÖNTEM: Çalışma, 01.10.2018–30.04.2019 tarihleri arasında, Sağlık Bilimleri Üniversitesi Ankara Numune Sağlık Uygulama ve Araştırma Merkezi Acil Tıp Kliniği'nde kontrollü, ileriye yönelik, klinik bir çalışma olarak yapıldı. Çalışmada hasta ve sağlıklı gönüllülerden alınan kanda, tiyol disülfid homeostazisinin değerlendirilmesi yapıldı. Çalışmaya acil servise başvuran travmatik hemorajik kanaması olan 18 yaş ve üstü, kadın-erkek 52 gönüllü hasta ve herhangi bir hastalığı olmayan sağlıklı gönüllü kişilerden oluşan 50 kişilik kontrol grubu olmak üzere toplam 102 kişi alındı.
BULGULAR: Hasta ve kontrol grubunda native tiyol, total tiyol, disülfid, disülfid/native tiyol, disülfid/total tiyol düzeyleri arasında anlamlı fark bulundu (sırasıyla, native tiyol, total tiyol, disülfid, disülfid/native tiyol ve disülfid/total tiyol p değerleri; 0.001>, 0.001>, 0.018, 0.002, 0.002). İkili grup karşılaştırmalarına göre; Evre 3 hemorajik şokta native tiyol ve total tiyol, Evre 1 ve 2 hemorajik şoka göre istatistiksel olarak anlamlı şekilde daha düşüktür. (Evre 1 ve Evre 3 hemorajik şok karşılaştırması; native tiyol için p değeri=0.001, total tiyol için p değeri=0.002) (Evre 2 ve Evre 3 hemorajik şok karşılaştırması; native tiyol için p değeri=0.009, total tiyol için p değeri=0.006). Total tiyol düzeyi hayatını kaybeden hastalarda yaşayanlara göre daha düşük bulundu (p=0.040).
TARTIŞMA: Bu çalışma ile tiyol disülfid homeostazisi verilerinin şok evresi ve mortalite ile korelasyon gösterdiği bulunmuştur. Tiyol disülfid homeostazisi değerlendirmesinin travmatik hemorajik şok hastalarında; hastalığın şiddeti, evrelemesi, prognozunu değerlendirmek ve tedavi yönetimi konusunda bize rehberlik edebileceği düşünülmektedir.
Anahtar sözcükler: Disülfid; nativ tiyol; şok evresi; total tiyol; travmatik hemorajik şok.

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