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ORIGINAL ARTICLE

The Relationship Between Serum Thiol Levels and Thiol/Disulfide Homeostasis with Head Trauma in Children

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SUMMARY

Background: **Oxidative stress may induce brain injury. Thiols are one of the most important antioxidant agents, and thiol/disulphide (SH/SS) homeo stasis is a novel oxidative stress marker. The goal of the study was to investigate the relationship of thiol levels and SH/SS homeostasis with head trauma in pediatric patients.**
Methods: **This prospective study was conducted in 85 consecutive pediatric patients aged < 18 years with isolated head trauma and 58 age- and gender-matched healthy controls in the Emergency Department (ED).**
Results: **The mean age was 4.40 ± 3.03 years for the patient group and 4.75 ± 1.81 years for the controls ($p > 0.05$). There were no significant differences in biochemical parameters including serum albumin, urea, alanine aminotransferase, total bilirubin, uric acid, high-sensitivity C-reactive protein (hs-CRP), and white blood cells (WBC) in the patient and control groups (for each, $p > 0.05$).**
The thiol (SH) level was significantly higher in the patient group than in the controls (388.83 ± 51.949 vs. 369.04 ± 37.62 µmol/L; $p = 0.009$). The total thiol (TT) level was somewhat higher in the patient group, but the difference was not significant (416.11 ± 47.29 vs. 405.08 ± 35.27 µmol/L; $p = 0.113$). The disulphide (SS) level was lower in the patient group ($p < 0.001$). The SS/SH and SS/TT ratios were significantly lower in the patient group, while the SH/TT ratio was significantly higher ($p < 0.001$).
Conclusions: **Analysis of serum thiol levels and SH/SS homeostasis might be useful in order to determine the head trauma in pediatric patients.**
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KEY WORDS

antioxidants, serum thiol levels, thiol/disulfide homeostasis, children, head trauma

INTRODUCTION

More than 1.7 million traumatic brain injuries occur in adults and children each year, with approximately 30% occurring in children. Brain injury is a significant cause of morbidity and mortality in pediatric trauma patients [1]. Brain injury after trauma may be primary or secondary. Primary brain injury is a direct consequence of the trauma. Secondary injuries include traumatic and hypoxic ischemia, infarction, secondary bleeding, and

diffuse brain edema. Secondary brain injury is caused by neuromodulators (components of the trauma response) and may have more serious consequences than primary injury [1,2]. Early identification of traumatic brain injury is crucial in decreasing the risk of severe brain injury [1]. Computerized tomography (CT) scans are recommended for evaluation of patients with head trauma. But, an increase in brain CT use which is expensive and associated with a high radiation dose has raised concerns regarding resource utilization and, more recently, the clinical consequences of radiation. Unnecessary brain CT scan in children may cause adverse effects of radiation, including brain cancer. Additionally, for brain CT, children may need sedation with life-threatening side effects possibly occurring due to use of agents. Therefore, it is important to identify brain injury before scheduling a CT for pediatric patients with head trauma admitted to the emergency department (ED). Thus, unnecessary CT could be avoided in head trauma patients without brain injury. The Glasgow Coma Scale (GCS) is used in hospitals and emergency services for the neurological evaluation of patients with head trauma. However, scoring can be difficult in pediatric patients because the sutures remain open in children, and lesions in the intracranial space can become quite large before neurological symptoms develop. In addition, a child's skull is extremely elastic and deforms under pressure, and trauma in children can cause significant deformities and tearing without a noticeable fracture [3]. Due to concerns about the use of brain CT and the limitations of clinical scoring systems in children, an objecttive, laboratory-based predictor is needed. No known biochemical parameter reflects brain injury in pediatric patients and no such parameter indicates whether brain CT is required.

Oxidant and antioxidant agents are released following head trauma. Serum thiols, which are organic compounds with sulfhydryl groups, constitute an important part of the antioxidant defense system. An imbalance between oxidants and antioxidants may lead to oxidative stress. Upon oxidative stress, disulphide bonds undergo reversible formation between protein thiols and low molecular weight ones and thus thiol/disulphide (SH/SS) homeostasis is sustained [3,4]. SH/SS homeostasis as a marker of oxidative status is an emerging novel biomarker. In this study, we explored the relationship of serum thiol levels and SH/SS homeostasis with head trauma in pediatric patients.

MATERIALS AND METHODS

This prospective study was conducted between August 2015 and January 2016 in the ED of a tertiary care university hospital. The subject group initially comprised 106 pediatric patients (age < 18 years) with isolated head trauma, who had no pathology other than head trauma, no systemic disease evident on clinical and laboratory examinations, and did not use any drug; however, 21 of these patients were later excluded because their blood samples underwent hemolysis. Thus, we ultimately enrolled 85 patients. The control group consisted of 75 age- and gender-matched healthy children aged < 18 years who had no complaints, no pathology evident on physical examination, no local or systemic disease apparent on clinical and laboratory examinations, and who did not use any drug. Of these, 17 patients were excluded because their blood samples underwent hemolysis. Thus, we ultimately enrolled 58 controls recruited. The patients and healthy volunteers were informed about the study protocol, and written consent was obtained from all participants' legal guardians (etc. family member). The study protocol was conducted in accordance with the Declaration of Helsinki.

Sample preparation

Venous blood samples of 4 mL were collected in tubes containing EDTA from patient and healthy volunteers and centrifuged at 1,500 rpm for 10 minutes, after family approval had been granted. The serum was stored at -80°C before biochemical analysis. Blood samples of the patients were taken immediately after arrival at the ED (within 24 hours of injury). Blood collection and examination extended over 90 days from the time of collection of the first sample.

Measurement of serum thiol levels and dynamic thiol/disulfide homeostasis

We used the novel automated method of Erel et al. [5] to evaluate plasma native thiol (-SH)/disulphide (-SS) homeostasis. In this technique, SS bonds are first reduced to reactive SH groups by sodium borohydride. The residual sodium borohydride is then consumed by the addition of formaldehyde, leaving only reduced SH group. Next, the level of native (non-reducing) SH group was measured by reaction with 5,5′-dithiobis-(2-nitrobenzoic) acid. The native SH level was subtracted from the total SH level and the number of SS bonds was half of the difference [5]. The SH, total thiol (TT), and SS levels were used to calculate the SS/SH, SS/TT, and SH/TT ratios.

Statistical analyses

Categorical variables were presented as number and percentages and numerical data as means and standard deviations. The statistical analyses were performed using SPSS ver. 21.0 (SPSS, Chicago, IL, USA) by the independent-samples *t*-test, analyses among demographic data, cross-tabulation, and frequency analysis as appropriate. Values of $p < 0.05$ were considered statistically significant.

Table 1. Clinical characteristics of 85 patients with isolated head trauma.

Clinical parameter		n (%)
Type of trauma	Fall	73 (85.9)
	Assault	7 (8.2)
	Traffic accident	5 (5.9)
Type of treatment	Medical	81 (95.3)
	Surgical	4 (4.7)
Outcome	Healed	7 (90.6)
	Restoration	7 (8.2)
	Died	1 (1.2)
GCS score	High	5 (5.9)
	Medium	16 (18.8)
	Low	64 (75.3)
Hospitalized		26 (30.6)
Outpatient		59 (69.4)

GCS - Glasgow Coma Scale.

Table 2. Characteristics of the pediatric patients with head trauma and controls.

Parameter	Patient (n = 85)	Control (n = 58)	p
Male/female, n	50/35	32/26	0.421
Age (years), mean ± SD	4.40 ± 3.03	4.75 ± 1.81	0.421
Urea (mg/dL), mean ± SD	22.72 ± 7.68	23.48 ± 5.39	0.488
ALT (U/L), mean ± SD	22.2 ± 19.35	18.74 ± 7.52	0.139
hs-CRP (mg/dL), mean ± SD	0.12 ± 0.38	0.0361 ± 0.16	0.065
WBC ($10^3/mm^3$), mean ± SD	14.43 ± 4.84	12.47 ± 3.51	0.196
Albumin (g/dL), mean ± SD	4.62 ± 0.3	4.59 ± 0.35	0.618
Total bilirubin (mg/dL), mean ± SD	0.30 ± 0.18	0.30 ± 0.2	0.909
Uric acid (mg/dL), mean ± SD	3.53 ± 0.91	3.62 ± 0.95	0.556

SD - standard deviation, ALT - alanine aminotransferase, hs-CRP - high-sensitivity C-reactive protein, WBC - white blood cell count.

Table 3. Serum thiol levels and ratios in the patient and control groups.

Parameter *	Patient (n = 85)	Control (n = 58)	p
SH (µmol/L)	388.83 ± 51.94	369.04 ± 37.62	0.009 *
TT (µmol/L)	416.11 ± 47.29	405.08 ± 35.27	0.113
SS (µmol/L)	13.64 ± 5.31	18.02 ± 6.42	< 0.001 *
SS/SH	3.66 ± 1.7	5.01 ± 2.25	< 0.001 *
SS/TT	3.37 ± 1.45	4.48 ± 1.74	< 0.001 *
SH/TT	93.26 ± 2.9	91.03 ± 3.48	< 0.001 *

*** Data shown as mean ± SD (standard deviation), SH - native thiol, TT - total thiol, SS - disulphide.**

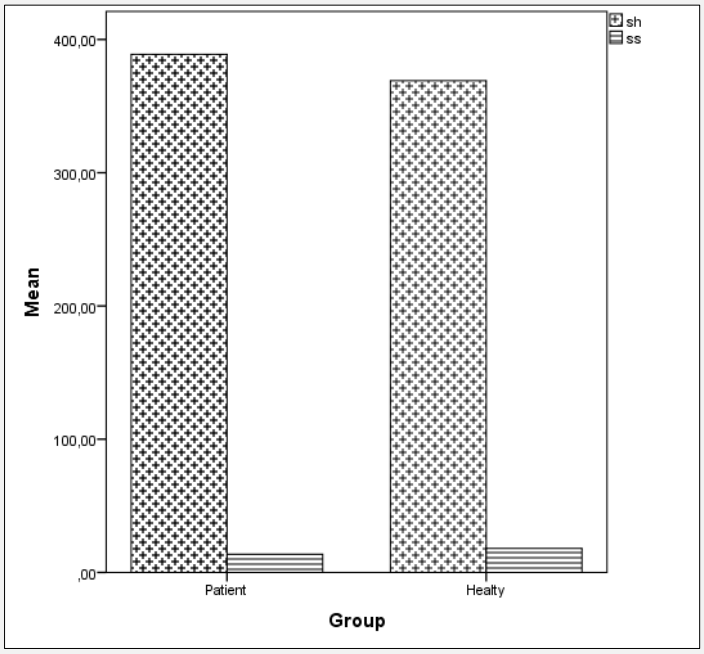

Figure 1. Native thiol (sh) and disulfide (ss) levels in the patient and control groups.

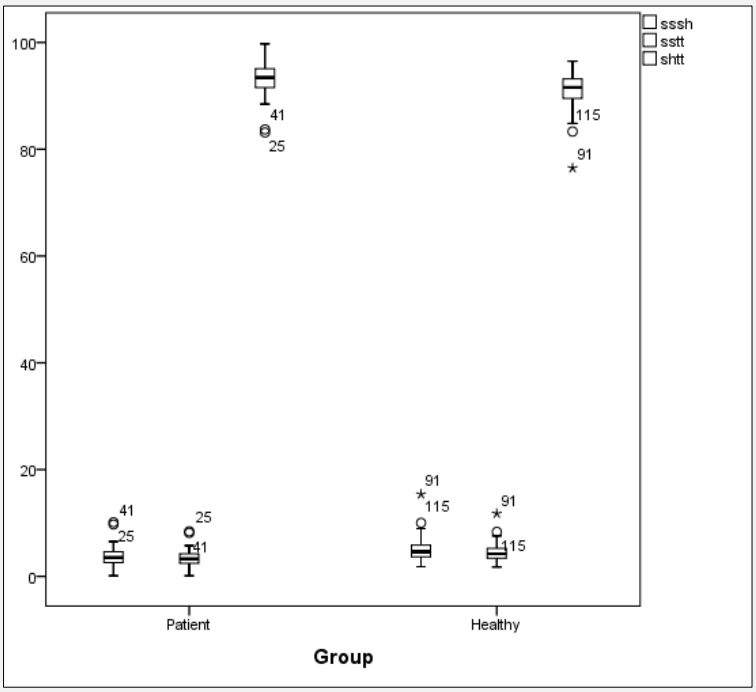

Figure 2. Comparison of the SS/SH, SS/TT, and SH/TT ratios in the patient and control groups.

RESULTS

The study included 85 patients with isolated head trauma and 58 healthy subjects as controls. Clinical characteristics of 85 pediatric patients with isolated head trauma are shown in Table 1. Of those patients, 73 (85.9%) had fallen or been dropped, 7 (8.2%) had been injured in the head directly (assault), and 5 (5.9%) had been involved in traffic accidents. Using the GCS, 5 (9.9%) patients had severe head trauma (GCS = 3 - 8), 16 (18.8%) had moderate head trauma (GCS = 9 - 12), and 64 (75.3%) had mild head trauma (GCS = 13 - 15). Of these cases, 59 (69.4%) were treated as outpatients, while 26 (30.6%) were hospitalized. Medical treatment was administered to 81 (95.3%) patients, while 4 (4.7%) required surgical treatment. Of the treated patients, 77 (90.6%) healed uneventfully and 7 (8.2%) had various neurological sequelae. The head trauma in 1.2% of the patients was due to primary or secondary pathologies. The gender and mean age of the participants with isolated head trauma and those of the control group were similar. In terms of gender, 50 patients were male (58.8%), 35 were female (41.2%), while 32 of the controls were male (55.2%) and 26 were female (44.8%). There was no significant difference in the male-to-female ratio ($p > 0.05$). The mean age of the patient group was 4.40 ± 3.03 years compared with 4.75 ± 1.81 years for the controls; the difference was not significant ($p > 0.05$). There were no significant differences in biochemical parameters such as the serum albumin, bilirubin, and uric acid levels in the patient and control groups $p > 0.05$). There was no significant difference in the high-sensitivity C-reactive protein (hs-CRP) or white blood cell (WBC) parameters ($p = 0.065$ and $p = 0.196$, respectively) (Table 2).

Table 3 lists the SH, TT, and SS levels, and the SS/SH, SS/TT, and SH/TT ratios for the patient and control groups. The SH level was significantly higher in the patient group than in the controls (388.83 ± 51.949 vs. 369.04 ± 37.62 μmol/L; $p = 0.009$; Figure 1). The TT level was somewhat higher in the patient group, but the difference was not significant (416.11 ± 47.29 vs. 405.08 ± 35.27 μmol/L; $p = 0.113$). By contrast, the SS level was lower in the patient group ($p < 0.001$) (Table 3). In the patients, the SS/SH ratio was 3.66 ± 1.7, the SS/TT ratio was 3.37 ± 1.45, and the SH/TT ratio was 93.26 ± 2.9; in the controls, the respective values were 5.01 ± 2.25, 4.48 ± 1.74, and 91.03 ± 3.48 (Figure 2). The SS/SH and SS/TT ratios were significantly lower in the patient group ($p < 0.001$), while the SH/TT ratio was significantly higher ($p < 0.001$).

DISCUSSION

Brain damage may develop, initially via induction of a post-traumatic inflammatory response. Inflammation commences with tissue infiltration by neutrophils. Free radicals and proteases released from activated neutrophils trigger brain edema by disrupting the blood-brain barrier. Free oxygen radicals damage macromolecules, including lipids, proteins, carbohydrates, and DNA. The greatest proportion of free radical-induced damage is thought to be due to hydroxyl radicals, which are the most toxic free radicals. After head trauma, neuronal nitric oxide synthase and inflammatory nitric oxide synthase disrupt mitochondrial function by generating free radicals [6-8].

Oxidative stress may induce brain damage and influence clinical outcome during the early posttraumatic period [3]. Previous studies evaluated the total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI) as biochemical indicators of oxidative stress in trauma patients. Gökdemir et al. [9] found that the TAS decreased significantly and the TOS and OSI increased significantly in patients with multiple blunt trauma injuries. Söğüt et al. [10] found that the TAS, TOS, and OSI differed significantly under conditions of varying oxidative stress in adult patients with head trauma. Kaya et al. [3] found that the TOS and OSI may be useful for predicting the prognosis in pediatric patients with head trauma in the early post-trauma period.

Thiols are antioxidant agents and react with free radicals to protect against tissue and cell damage caused by reactive oxygen species including oxidant agents. Most of the plasma thiol pool consists of proteins, with small proportions of low-molecular-weight thiols such as cysteine, cysteinylglycine, glutathione, homocysteine, and γ-glutamyl cysteine. SH groups are oxidized by oxidants in the medium, and the resulting SS bonds are then reduced back to SH groups, maintaining the SH/SS balance. This process is the earliest manifestation of free radical-mediated protein oxidation. The loss of protein SH groups is the principal cause of structural and functional changes. The dynamic SH/SS equilibrium plays critical roles in antioxidant defense, detoxification, apoptosis, the regulation of enzyme activities, transcription, and cellular signal transduction. SH groups can form SS groups and other oxidized products. SS bonds are covalent and are also called SS bridges. Oxidation of cysteine residues under conditions of oxidative stress can trigger reversible SS formation between low-molecular-weight SH and protein SH groups. The resulting SS bonds may then be reduced to SH groups, thereby maintaining the dynamic SH/SS balance [11,12].

No study has yet explored the relationship between pediatric head trauma and the SH, TT, SS levels, and SH/SS balance, but a few studies have explored these levels and balance in patients with other diseases. Türkoğlu et al. [13] found that the SH and TT levels and the SH/TT ratio were significantly lower in patients with central serous chorioretinopathy than in controls, while the SS/SH and SS/TT ratios were significantly higher in the patients. Additionally, the SH levels have been measured in patients with pre-eclampsia [14], autoimmune subclinical hypothyroidism [15], acute myocardial infarc-

tion [16], and acute ischemic stroke [17].
We found significant increases in the SH levels and SH/TT ratio in pediatric patients with isolated head trauma. Additionally, the TT level was somewhat higher in the patient group than in the controls, but the difference was not significant. The SS level and the SS/SH and SS/TT ratios were significantly lower in the patient group than in the controls. Therefore, in pediatric patients with isolated head trauma, the SH/SS equilibrium did not shift toward SS bond formation. According to the hypothesis in a study of Erel et al. [5], the SH/SS balance did not shift in the SS direction because the trauma did not exceed the oxidative stress threshold. On the contrary, a significant shift in the opposite direction was evident. We believe that the SH/SS balance does not shift in the SS direction if the blood-brain barrier is not damaged. A limitation of the present study is that the correlations among injury severity, cranial lesions and neurological outcomes with serum thiol levels and SH/SS homeostasis were not investigated. Further studies are required to better explain SH/SS homeostasis in pediatric patients with head trauma and the associations with the neurological findings.

CONCLUSION

The SH level and SH/TT ratio were higher in the patient group than in the controls while the SS level, SS/SH, and SS/TT ratios were lower in the patient group. Analysis of serum thiol levels, ratios, and SH/SS homeostasis might be useful in order to determine brain damage in pediatric patients with head trauma.

Human Subject Protections:

The study was performed in compliance with the World Medical Association Declaration of Helsinki on Ethical Principles for Medical Research Involving Human Subjects.

Declaration of Interest:

No conflict of interest was declared by the authors and the authors declared that this study has received no financial support.

References:

1. Morrissey K, Fairbrother H, Vazquez MN. Severe traumatic brain injury in children: an evidence-based review of emergency department management [digest]. Pediatr Emerg Med Pract. 2016 Oct 22;13 (10 Suppl Points & Pearls):S1-S2 (PMID: 28745856).

2. Wilson JR, Green A. Acute Traumatic Brain Injury: A Review of Recent Advances in Imaging and Management. Eur J Trauma Emerg Surg. 2009;35(2):176 (PMID: 26814773).

3. Kaya H, Sogüt O, Gokdemir MT, Albayrak L, Taskın A. The role of oxidative status in initial evaluation of paediatric patients with graded traumatic brain injury. Hong Kong J Emerg Med 2013; 20(4):226–32. http://www.hkjem.com/2013-july/original-article/role-oxidative-status-initial-evaluation-paediatric-patients-graded

4. Altıparmak IH, Erkuş ME, Sezen H, et al. The relation of serum thiol levels and thiol/disulphide homeostasis with the severity of coronary artery disease. Kardiol Pol. 2016;74(11):1346-53 (PMID: 27221962).

5. Erel O, Neşelioğlu S. A novel and automated assay for thiol/disulphide homeostasis. Clin Biochem 2014;47:326-32 (PMID: 25304913).

6. Moriarty-Craige SE, Adkison J, Lynn M, et al. Antioxidant supplements prevent oxidation of cysteine/cystine redox in patients with age-related macular degeneration. Am J Ophthalmol 2005; 140:1020-6 (PMID: 16376645).

7. Olusi SO. Obesity is an independent risk factor for plasma lipid peroxidation and depletion of erythrocyte cytoprotective enzymes in humans. Int J Obes Relat Metab Disord 2002;26:1159-64 (PMID: 12187391).

8. Ray G, Batra S, Shukla NK, et al. Lipid peroxidation, free radical production, and antioxidant status in breast cancer. Breast Cancer Res Treat 2000;59:163-70 (PMID: 10817351).

9. Gökdemir MT, Sogüt O, Kaya H, et al. Role of oxidative stress in the clinical outcome of patients with multiple blunt trauma. J Int Med Res 2012;40:167-73 (PMID: 22429356).

10. Söğüt Ö, Kaya H, Gökdemir MT, et al. Early oxidative status in adult patients with isolated traumatic brain injury. Turk J Med Sci 2012; 42 (6): 1010-9. http://journals.tubitak.gov.tr/medical/abstract.htm?id=13101

11. Türkcü FM, Yüksel H, Sahin A, et al. Serum dehydroepiandrosterone sulphate, total antioxidant capacity, and total oxidant status in central serous chorioretinopathy. Graefes Arch Clin Exp Ophthalmol2014;252:17-21 (PMID: 23749383).

12. Cremers CM, Jakob U. Oxidant sensing by reversible disulfide bond formation. J Biol Chem 2013;288(37):26489-96 (PMID: 23861395).

13. Turkoglu EB, Dikci S, Çelik E, et al. Thiol/Disulfide Homeostasis in Patients with Central Serous Chorioretinopathy. Curr Eye Res. 2016;41(11):1489-91 Epub 2016 Mar 22 (PMID: 27002242).

14. Korkmaz V, Kurdoglu Z, Alisik M, et al. Impairment of thiol-disulfide homeostasis in preeclampsia. J Matern Fetal Neonatal Med. 2016;29(23):3848-53 (PMID: 26828694).

15. Ates I, Altay M, Yilmaz FM, et al. Dynamic thiol/disulfide homeostasis in patients with autoimmune subclinical hypothyroidism. Endocr Res. 2016 Nov;41(4):343-349 (PMID: 27045442).

16. Kundi H, Ates I, Kiziltunc E, et al. A novel oxidative stress marker in acute myocardial infarction; thiol/disulphide homeostasis. Am J Emerg Med 2015;33:1567-71 (PMID: 26143314).

17. 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 (PMID: 26782823).