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# Assessment of thiol disulfide homeostasis in isolated head injury

Thiole disulfite homeostasis in isolated head injuries

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**Abstract**
Aim: This study aimed to differentiate traumatic brain injury (TBI) and non-traumatic brain injury (NTBI) among patients presenting to the emergency department with isolated head trauma. For this purpose, we investigated whether the combined use of the parameters of thiol/disulfide homeostasis, an oxidative stress marker, and IMA (Ischemia Modified Albumin) was useful in differentiating the two conditions.
Material and Methods: This study was prospectively conducted on 92 patients who presented to the emergency department between 01.06.2018 and 01.01.2019, and 40 healthy subjects as controls. Thiol/disulfide homeostasis parameters (Thiol, disulfide), are the oxidative stress markers measured by a novel method developed by Erel and Neşelioğlu, and IMA were studied in the patient and control groups.
Results: NT and TT values were significantly lower in the TBI group than in the NTBI group (p <0.001, <0.001, respectively). Disulphide, IMA, and index1 and index 2 values were significantly higher in the TBI group compared to the NTBI and the control groups (p =0.019, <0.001, < 0.001, and < 0.001, respectively).
Discussion: The results of the present study suggest that these easy-to-perform, cheap, and automated biomarkers may help make a differential diagnosis between the two conditions.

**Keywords**
Isolated Head Trauma, Traumatic Brain Injury, Thiol/Disulfide Homeostasis

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## Introduction

Traumatic brain injury (TBI) is a leading cause of mortality and morbidity. From the standpoint of public health, it has a significant share among deaths under the age of 45 years [1,2]. The incidence and mortality and morbidity due to head trauma have been increasing as a result of developing technology and social developments [3]. Primary brain injury occurs as a result of trauma and physical effect on the central nervous system (CNS). This results in scalp injury, skull fracture, shrinkage, brain laceration, diffuse axonal injury, and intracranial bleeding (epidural, subdural, intracerebral). Post-traumatic injury depends on trauma mechanism and trauma severity [4]. After a primary injury, neurophysiological and biochemical mediators initiate secondary cellular injury [5] Formation of reactive oxygen species and lipid peroxidation (LP) causes stimulatory glutamate and aspartate release, calcium influx to the cell, and formation of eicosanoids. This impairs cell membrane permeability and leads to secondary cellular injury. Many studies to date have shown that oxygen free radicals play an important role in the occurrence of secondary injury and cause neurotoxicity [6,7]

The imbalance between the cellular production of oxygen radicals and cellular defense mechanisms against them is called oxidative stress [8]. The latter begins right after TBI and initiates a cascade that results in neuronal dysfunction and death. It plays an important role in secondary injury and is responsible for morbidity and mortality after TBI. However, the underlying molecular mechanisms are complex and have not been completely understood [1] Oxidative stress, i.e an imbalance between oxidizing agents and antioxidants, contributes to the pathogenesis of TBI [9]

Until recently, only one aspect of the thiol-disulfide balance has been measured. However, both sides of the balance can be measured and thiol-disulfide status can be completely evaluated using novel test methods [10]

The main aim of the present study was to investigate whether thiol/disulfide homeostasis parameters, which are among the oxidative stress markers, are useful for the differential diagnosis of TBI and NTBI among patients presenting with isolated head trauma.

## Material and Methods

This open-label, prospective, controlled study enrolled a total of 132 subjects, which consisted of 92 patients and 40 healthy volunteers. The patients were grouped into two as traumatic brain injury (TBI) (n=47) and non-traumatic brain injury (NTBI) (n=45) groups. The study was conducted at the Emergency Department Training and Research Hospital between 01.06.2018 and 01.01.2019 and was approved by the Local Ethics Committee. All patients read the informed consent form and gave written informed consent for study participation. Patients older than 18 years of age who presented to the emergency department with isolated head trauma were enrolled. Patients with isolated head trauma were categorized into two groups, namely the traumatic brain injury group, and the non-traumatic brain injury group. Patients who were pregnant, who refused to participate in the study, and who were immunocompromised were excluded.

Blood samples were taken within the first hour of emergency department admission, before starting any medication. The levels of the parameters used to evaluate THD were measured in an automatic analyzer by a novel spectrophotometric method [10]. This method quantifies native thiol (NT), total thiol (TT), and disulfide (D) levels. Then, the ratios of these three parameters to each other are calculated (index 1: D/NT, index 2: D/TT, index 3: NT/TT). Serum Ischemia Modified Albumin (IMA) levels were quantified by a previously described colorimetric experimental technique [11]. In short, it involved the admixture of the serum in question at an amount of 200 uL, and then 0.1% CoCl2 50 uL and incubated at 37° C in dark, followed by a 10-minute addition. It was followed by the addition of 50 uL dithiothreitol as a coloring agent. One milliliter of 0.9 % NaCl was added 2 minutes after incubation and the reduction agent and the blank control were prepared similarly. Absorbance spectrophotometry was used at 470 nm to obtain readings. IMA was recorded as the absorbance unit. Both samples were measured twice and their mean value was reported. Thiol/disulfide analysis results were compared in the patient volunteers.

### *Statistical analysis*

Statistical analyses of the study data were performed with SPSS 16.0 for the Windows software package. The normality of the data was tested using the Kolmogorov-Smirnov test. Normally distributed variables were reported as mean±standard deviation, and non-normally distributed ones as median (min-max). Three independent groups were compared using the Kruskal-Wallis test for variables without normal distribution and One-way ANOVA with posthoc Bonferroni test for normally distributed variables. Receiver Operating Characteristics (ROC) curves with area under the curve, sensitivity, and specificity values were drawn to determine the predictive ability of oxidative stress parameters for disease prediction. Statistical significance was set at $p<0.05$.

## Results

This study enrolled 47 patients with TBI, 45 patients with NTBI, and 40 healthy volunteers. There was no significant difference between the groups regarding sex distribution whereas they were significantly different concerning age (Table 1).

NT, TT, Disulfide, IMA, index 1, and index 2 were significantly different but there was no significant difference concerning index 3. The subgroup analyses showed that the difference originated from the TBI group. NTBI and control groups were not significantly different. NT and TT were significantly lower in the TBI group compared to the NTBI group. Disulfide, IMA, index 1 and index 2 values were significantly higher in the TBI group compared to NTBI and control groups (Table 2).

ROC analyses were performed and the area under the curve

**Table 1.** Demographic features of the groups

| Demographics | Control (n=40) | NTBI (n=45) | TBI (47) | p-value |
|---|---|---|---|---|
| Age-median (min-max) | 36 (18-59) | 38 (20-90) | 45 (20-60) | 0.036 |
| Gender (female/male) | 16/24 (40/60 %) | 23/24 (48,9/51,06 %) | 14/31 (31,11/68,8) | 0.267 |

TBI: Traumatic brain injury ; NTBI: Non-traumatic brain injury

**Table 2.** Thiol/disulphide homeostasis parameters (Control, TBI, NTBI groups)

| NTBI | | | | | | | |
|---|---|---|---|---|---|---|---|
| | TBI n=45 | | TBI n=47 | | Control N=40 | | p-value |
| | Mean ±SD | Med (Min Max) | Mean±SD | Med (Min Max) | Mean±SD | Med (Min Max) | |
| NT* | 418,0±62,1 | 408,4 (251,8-534,6) | 363,6±75,1 | 358,1 (214,0- 549,3) | 447,7±48,7 | 445,6 (310,4-550,0) | <0,001 |
| TT* | 462,7±71,5 | 459,6 (303,9-597,4) | 406,6±84,2 | 402,9 (243,0 -602,9) | 490,5±57,1 | 480,5 (344,4-619,7) | <0,001 |
| DISULPHIDE | 13±8 | 12 (0-31) | 18±9 | 17 (2-36) | 14±8 | 12(2-41) | 0,019 |
| IMA(ABSU) | 73,5±5,1 | 72,7 (64,6-81,9) | 77,3±5,8 | 78,0 (60,0-83,9) | 59,0±7,5 | 55,4(50,0-83,6) | <0,001 |
| Index1* | ,03±,02 | ,03 (0,00-,12) | ,05±,03 | ,05 (,00-,14) | ,03±,02 | ,03 (,00-,09) | <0,001 |
| Index2 | ,03±,02 | ,03(0,00-,10) | ,05±,02 | ,05 (,00-,11) | ,03±,01 | ,03 (,00-,08) | <0,001 |
| Index3 | ,91±,08 | ,93 (,71-1,02) | ,90±,08 | ,90 (,59-1,18) | ,92±,09 | ,94 (,60-1,06) | 0,067 |

TBI : Traumatic brain injury; NTBI :Non-traumatic brain injury, Med: Median; SD: Standard deviation; NT: native thiol; TT: total thiol; D: disulfide; Index 1: D/NT; Index 2: D/TT; Index 3: NT/TT; IMA: Ischemia Modified Albumin; ABSU: Absorbance units , *One way-ANOVA and Bonferroni posthoc test were used, for others: Kruskal-Wallis test

**Table 3.** ROC analysis

| Variable(s) | Area | Std. Error | Asymptotic 95% Confidence Interval | |
|---|---|---|---|---|
| | | | Lower Bound | Upper Bound |
| NT | 0,732 | 0,053 | 0,628 | 0,836 |
| TT | 0,705 | 0,055 | 0,597 | 0,813 |
| Disulfide | 0,638 | 0,058 | 0,524 | 0,752 |
| IMA (ABSU) | 0,711 | 0,054 | 0,605 | 0,818 |
| Index 1 | 0,694 | 0,056 | 0,585 | 0,803 |
| Index 2 | 0,712 | 0,055 | 0,604 | 0,819 |
| **Cut-off values for Native Thiol (diagnostic evaluation for the TBI)** | | | | |
| | Cut-off | Sensitivity | 1-Specificity | |
| Native Thiol | 461,7 | 0,915 | 0,756 | |
| | 435,45 | 0,851 | 0,578 | |
| | 405,65 | 0,787 | 0,489 | |
| | 403,65 | 0,787 | 0,422 | |
| | 390,6 | 0,681 | 0,267 | |
| | 337,45 | 0,404 | 0,089 | |
| | 293,6 | 0,213 | 0,022 | |
| | 289 | 0,191 | 0,022 | |

TBI: Traumatic brain injury; NTBI NT: native thiol; TT: total thiol; D: disulfide; Index 1: D/NT; Index 2: D/TT; IMA: Ischemia Modified Albumin; ABSU: Absorbance units

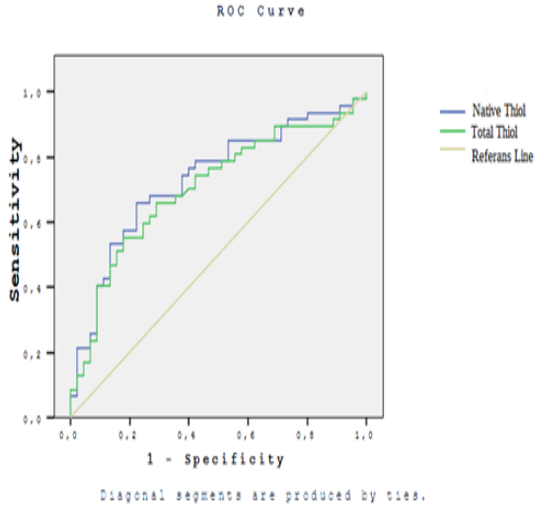


**Figure 1.** ROC analysis and area under the curve for NT, TT (diagnostic evaluation of TBI)

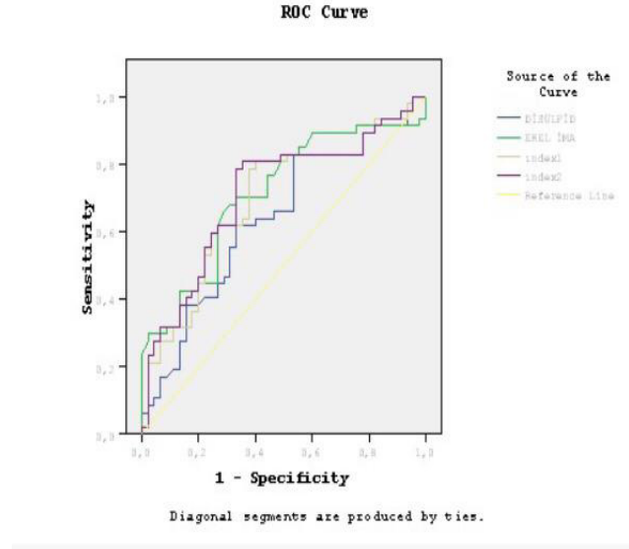


**Figure 2.** ROC analysis and area under the curve for Disulfide, IMA, Index 1, Index 2 (diagnostic evaluation of TBI)

was calculated for NT, TT, Disulfide, IMA, index 1, and index 2 in the TBI and NTBI groups. The area under the curve was the largest for NT, with the latter's sensitivity and specificity being presented below. To give an example, traumatic brain injury was present at a rate of 100% for an NT value below 461.70, while for values above that level one must assume that traumatic brain injury is not present at a rate of 24.40%. One must assume that traumatic brain injury is present at a rate of 78.70% for NT values below 403.65 and absent at a rate of 57.80% for NT values above 403.65 (Figures 1-2 and Table 3).

**Discussion**

Our study examined the change of oxidative stress parameters after isolated head trauma among patients presenting with TBI. Oxidative stress occurs after secondary injury and leads to permanent brain injury [12]. Therapeutic interventions during secondary brain injury are essential. Many hallmarks are exhibited during delayed secondary CNS damage, mainly including mitochondrial dysfunction, Wallerian degeneration of axons, excitotoxicity, oxidative stress, and eventually neuronal death and overactivation of glial cells [13]. Cerebral endogenous antioxidant defense mechanisms neutralize superoxide radicals and form enzymes such as catalase activity, superoxide dismutase (SOD), and glutathione peroxidase (GPx), all of which protect cells against OS-induced injury [14].

The plasma thiol pool is one of the antioxidant mechanisms. Thiols are oxidized by oxidants and form disulfide bridges. The latter may be reduced to thiol groups. This dynamic thiol/disulfide hemostasis has a vital role in antioxidant defense [10]. Erel and Neşelioğlu measured thiol/disulfide homeostasis with the automatic colorimetric method for the first time in the literature; they revealed that disulfide levels increased in smokers and patients with diabetes, obesity, and pneumonia and decreased in some cancer types [10]. We demonstrated that thiol/disulfide homeostasis parameters did not differ between healthy volunteers and the NTBI groups, but they were significantly reduced in the TBI group compared to the NTBI and control groups. Disulfide, IMA, index 1, and index 2 values increased to a greater degree in the TBI group compared to the NTBI and control groups. We believe that the significant reduction in NT and TT levels in the TBI group compared to the control group and healthy volunteers occurred as a result of an increased oxidative burden. A significant increase in disulfide level occurred, which we believe occurred by the reduction of thiols. Significant increases were detected in index1 and 2. We believe that the significant reductions in NT and TT levels occurred as a result of an increased oxidative burden; hence, an expected increase in Disulfide level occurred that we attributed to thiols' reduction.

Kavaklı et al. enrolled adult patients presenting to the emergency department with isolated blunt traumatic brain injury and healthy adults as controls [15]. They studied serum oxidant status by analyzing the Total Oxidation Status (TOS) in patients with traumatic brain injury and healthy subjects. Serum antioxidant status was analyzed by measuring Total Antioxidant Status (TAS). They also calculated Oxidative Stress Index (OSI). TOS and OSI levels were increased in the patient group compared to the controls. Among patients that ultimately died, OSI, TOS, and TAS were higher. The authors suggested that oxidative stress parameters may be valuable prognostic markers and concluded that oxidative stress parameters may offer an opportunity for assessing the clinical severity of traumatic brain injury and predicting its outcome. We also demonstrated that NT and TT levels significantly decreased due to increased oxidative stress burden in the TBI group than in the NTBI and control groups. In addition, disulfide levels significantly increased as a result of thiols' reduction.

De-Sheng et al. reported that Thioredoxin (TRX), a powerful antioxidant, was higher in patients with head trauma compared to controls [16]. They observed a drop in Glasgow Coma Score as the TRX level increased. They also found a correlation between TRX and mortality rate. They concluded that TRX may be used as a prognostic marker among patients with TBI. Our study detected a significant increase in IMA and disulfide levels in the TBI group. While an increase in IMA and Disulphide levels occurred in the TBI group, it was absent in the NTBI group. Therefore, we think that these parameters, in combination with the clinical presentation, can be used as a discriminator in making decisions for radiological tests.

Lilios et al. examined glutathione peroxidase, which is an antioxidant molecule, in pediatric patients presenting with multi-trauma. They reported a significant drop in antioxidant levels in the first three days followed by a rise starting on the 7th day [17]. Our study similarly showed reduced levels of antioxidant TT and NT in the TBI group. Our study only took into consideration the admission levels of these parameters. We believe that intermittent monitorization of NT and TT can be used to correlate them with clinical status.

Şahin A et al. studied IMA levels among patients who presented to the emergency department with a headache. They observed that migraine was the most common headache etiology (32.6%). A comparison of the study groups concerning serum IMA levels revealed no significant differences between the primary and secondary headache groups, between life-threatening and non-life-threatening headache groups, and between migraine, cluster, and stress-type headache subgroups within the primary headache group ($p>0.05$) [18]. In our study, it was significant

in the TBI group compared to the NTBI and control groups. We believe that the mechanism underlying that finding is free radicals emerging in intracranial pathologies, especially secondary to ischemia, reperfusion, and hemorrhage passing to the systemic circulation through the blood-brain barrier, which leads to an increased IMA production. This suggests that this parameter can be used to indicate brain injury without needing any imaging study.

Gündüz et al. compared IMA levels of 43 patients with ischemic infarction, 11 patients with intraparenchymal hemorrhage, 52 patients with SAH, and 43 healthy controls [19]. Radwan et al. increased IMA levels in 49 patients with TBI who were admitted to the intensive care unit. Of the 49 patients with heightened IMA, 22 had a decrease in IMA, and 27 had an increase in 24 hours. IMA levels were higher in deceased patients than in survivors [20]. That study is in agreement with our study. IMA level increases in intracranial pathologies.

We did not come across any study that studied a combination of thiol/disulfide homeostasis and IMA in isolated head trauma. We believe that studying a combination of thiol/disulfide homeostasis parameters and IMA would be beneficial for making a differential diagnosis between TBI and NTBI. We believe that, in this way, unnecessary radiological evaluation can be avoided. We are of the opinion that this subject may also guide therapy and should be studied by larger studies.

### *Limitations*

The main limitation of our study is the small sample size. In isolated head trauma, one must not depend solely on a single biochemical test to detect brain injury and make correlations with clinical signs and other radiological studies. Thiol/disulfide homeostasis parameters and IMA should be evaluated in combination with other clinical and radiological parameters.

### *Conclusion*

Oxidative stress plays an important role in the pathogenesis of isolated head trauma. Pathogenesis was revealed by measuring thiol/disulfide homeostasis and IMA quantitatively with the technique developed by Erel and Neşelioğlu. The present study numerically demonstrated the difference between the groups with and without brain injury concerning oxidative stress levels using the above technique. Differences in the patient groups compared to the control group suggest that these biomarkers may aid in making a diagnosis of TBI and NTBI. In conclusion, we think that measured with an easy, cheap, and automated technique, these biomarkers would aid in making the differential diagnosis after being tested by larger studies. We also believe that studies using thiol/disulfide and IMA levels in homogenous patient groups would serve both to make a differential diagnosis and to avoid unnecessary radiological studies.

***Scientific Responsibility Statement***

*The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.*

***Animal and human rights statement***

*All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.*

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***Conflict of interest***

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