

# Evaluation of Dynamic / Thiol Disulfide Balance and İschemia Modified Albumin in Children with Trauma

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## Abstract

**Introduction:** Trauma is the most common cause of death in childhood. Tissue damage, ischemia-reperfusion injury and inflammatory response are mainly responsible for increasing serbest oxyigen radicals. In this study, we aimed to investigate the use of thiol-disulfide and ischemia-modified albumin levels as a diagnostic laboratory parameter in trauma children. **Method:** Of 202 children, 101 of them were hospitalized in pediatric surgical intensive care unit with trauma and 101 of others were healthy children. Levels of native thiol (-SH), total thiol (SH + SS), dynamic disulfide (SS), dynamic disulfide (SS) / total thiol (SH + SS), albumin and ischemic modified albumin (IMA) were measured from the sera of patients and healthy volunteers. For statistical analyses, SPSS 17.0 was used. Mann-Whitney U and paired correlation test were used where appropriate.  $p < 0.05$  was considered significant. **Results:** The mean age of the patients in the trauma group (Boys: 61 Girls: 40) was 7,88 years and the control group was 8,00 years. In trauma group, 86 of children were exposed to blunt trauma, 15 of children had penetrating trauma and 54 patients had multiple trauma. Surgical procedures were performed in 17 patients. In the trauma group, native thiol, total thiol, dynamic disulfide / total thiol, albumin and IMA levels were significantly lower than that of control ( $p < 0.001$ ) and their dynamic disulfide ( $p = 0.001$ ) was higher compared to the control. There was no difference thiol-disulfide parametres in trauma groups sub-division as surgery( $n=17$ ) vs. follow-up( $n=84$ ) groups or multiple trauma( $n=54$ ) vs. isolated organ trauma( $n=47$ ) groups, or penetrating( $n=15$ ) or blunt trauma( $n=86$ ) groups. **Conclusion:** Thiol-disulfide balance and IMA levels show changes in favor of oxidative stress in children with trauma, however, it cannot be used as a laboratory marker that helps to show the system and organ affected by the trauma and to decide the surgical intervention.

## 1.INTRODUCTION

It is known that oxidative stress increases as a result of the activation of neutrophils and macrophages and overproduction of reactive oxygen species (ROS) in inflammatory events [1-3]. Damage due to ROS is prevented by enzymatic or nonenzymatic antioxidant mechanisms including super-oxide dismutase, catalase and glutathione S-trans enzyme systems, and important biological thiols such as glutathione, cysteine, homocysteine, N-acetyl-cysteine and gamma glutamine.

Thiol is an organic compound and contains a -SH group that plays a critical role in the prevention of oxidative stress in cells. SH-containing amino acid groups in proteins are primary targets of ROS. In the same medium with ROS, the -SH groups are oxidized and form reversible disulfide bonds. The loss of thiol groups is the main molecular mechanism that results in structural and functional changes in proteins. Antioxidants and in particular thiol groups that try to prevent the destructive effects of free radicals cannot maintain plasma

and tissue levels during these interactions [4]. However, the resulting disulfide bonds can be reduced to the thiol groups by the cellular reduction effects of some antioxidants and thus the thiol/disulfide homeostasis is preserved [5]. The state of the dynamic thiol-disulfide balance plays a critical role in antioxidant protection, detoxification, signal transduction, apoptosis, the regulation of enzymatic activity, transcription factors and cellular signaling mechanisms [6]. In addition, distortions in the dynamic thiol-disulfide balance has been shown to play a role in the pathogenesis of many diseases such as diabetes, cardiovascular diseases, cancer, rheumatoid arthritis, chronic kidney disease, AIDS, Parkinson's disease, Alzheimer's disease, Friedrich's ataxia, multiple sclerosis, amyotrophic lateral sclerosis and liver diseases [7]. Therefore, determination of the dynamic thiol/disulfide balance may provide valuable information regarding various normal or abnormal biochemical processes [7]. A number of studies have shown the predictive value of thiol/disulfide hemostasis in organ ischemia. However, measurement of plasma/serum thiol-disulfide balance by colorimetric method has been made possible by the method developed by a Turkish scientist for the first time in the world [7].

Ischemia-modified albumin (IMA) is a FDA-approved test among the newly investigated cardiac markers [8]. The principle of the test is based on measuring the cobalt binding capacity of albumin, leading to chemical changes in the albumin during oxidation. This new albumin molecule is also called ischemia-modified albumin. The formation of this new albumin molecule, which has lost its ability to cobalt, is one of the earliest markers of ischemia [9]. However, recent studies suggest that IMA, which stands out as a marker of cardiac ischemia, may increase in different pathologies [10-12].

Trauma is one of the causes of admission to the emergency department and is the most common cause of death in childhood [13]. Both our country and the United States, trauma locates in the second place after the infectious diseases in the applications to the emergency services [14]. Trauma management should be fast and dynamic because it has a high mortality and morbidity. In all traumas, ABC algorithm should be started from the first encounter. The most common cause of trauma is traffic accidents. This is followed by fall from height. In children, blunt abdominal trauma is the most common trauma of the spleen and liver. Penetrating traumas in children constitute 10% of all traumas. However, mortality is higher in these patients. Gunshot wounds and sharp stab wounds are the most common causes. In the penetrating injuries in children, the most damaged abdominal organs are liver (28.8%), bowel (26%), abdominal large vessels (24.9%), diaphragm (22%), kidneys (11.3%), spleen (9.6%) and esophagus (9.6%), respectively [15].

### 1.1. Trauma and Oxidative stress

Trauma leads to oxidative stress by increasing the production of SORs and SARs due to the activation of polymorphonuclear cells and neutrophils stemming mainly from tissue damage, ischemia-reperfusion injury and consequently inflammatory response. It is known that oxidative stress is increased as a result of the activation of neutrophils and macrophages and over-production of SOR in inflammatory events [16,17,18]. In inflammation, SORs produced by polymorphonuclear leukocytes lead to lipid peroxidation in cell membranes. These changes in the cell membrane cause an increase in microvascular permeability, intestinal edema, infiltration of inflammatory cells, activation of neutrophils and cell death [17]. In literature, it has been shown in studies conducted by adult patients and animals that the head injury [19-22], burn [23], bone fractures [24], liver damage [25], kidney damage [26], lung injury [27] and similar traumas lead to distortion in oxidative balance resulting in oxidative stress. However, there are very few studies in children [28]. In traumatized children, the development of a test that could show the affected system and organ from trauma more quickly and easily and assist in deciding on surgical intervention would help both early diagnosis and early surgical intervention. In this study, we aimed to investigate the use of thiol-disulfide and ischemia-modified albumin levels as a diagnostic laboratory parameter in trauma children.

## 2. MATERIAL and METHODS

Of 101 children who were hospitalized between 1<sup>th</sup> April 2017 and 1<sup>th</sup> June 2018 in pediatric surgical intensive care unit due to trauma and 101 other children admitted by circumcision or incarcerated inguinal hernia were included to this study. The study was conducted prospectively in our hospital. The study permission was obtained from the ethics committee of the Ankara Child Health and Diseases Hematology Oncology Training

and Research Hospital (decision no. 2017/05 dated 27/03/2017). Informed consent form was obtained from the parents of both groups' children included to the study. The cases with trauma were compared with the control group in terms of thiol-disulfide levels and IMA. On the 2nd and 5th days of hospitalization, 2 mL of venous blood was taken to the yellow blood tube (BD Vacutainer plastic SST II tube ®) from children with trauma. Blood samples collected were centrifugated at 3600 rpm for 10 minutes in the biochemistry laboratory and 2 cc serum was obtained and stored at -80°C. After all the samples were collected, all of them were thawed at the same time and the blood thiol-disulfide parameters were determined by Erel & Neselioglu with the newly developed automatic measurement method [7] and the IMA levels were determined by Bar-Or et al. [29] method via the Roche Hitachi Cobas c501 automatic analyzer. The levels of native thiol (-SH), total thiol (SH + SS), dynamic disulfide (SS), dynamic disulfide (SS) / total thiol (SH + SS), albumin and ischemic modified albumin (IMA) were measured from the sera of patients and control group.

**2.1.Statistical analysis:** Statistical analysis was performed using SPSS 17.0 version (Chicago Inc., 2008). Shapiro-Wilk test was used to determine normality of the continuous variables. Numerical variables were controlled by analytical methods. Total thiol, native thiol (SH), dynamic disulphid (SS) and dynamic disulphid (SS) / total thiol (SS + SH), albumin and IMA values did not fit the normal distribution. For comparison, variables were analyzed by Mann-Whitney U and paired correlation test.  $p < 0.05$  was considered significant.

### 3. RESULTS

In this study, 101 children with trauma were evaluated and their variables were compared with 101 healthy children.

#### 3.1. Demographics of the groups

The mean age of the patients in the trauma group (boys: 61 girls: 40) was 7.8 years (standard deviation = 4.77, range = 0.8-17 years), and the control group 8 years (standard deviation = 3.9, range = 1-17 years). 86 of the cases in the trauma group were exposed to the blunt force, 15 to penetrating trauma, and 54 patients had multiple trauma. Surgical procedures were performed in 17 patients. The organs and systems of the cases affected by trauma were shown in Table 1. The most commonly affected organ was the spleen (n = 37).

#### 3.2.Laboratory findings

The trauma and control groups' blood parameters of native thiol (SH), total thiol (SH + SS), dynamic disulfide (SS), dynamic disulphid (SS) / total thiol (SS + SH) %, ischemic modified albumin (IMA) and albumin levels were shown in Table 2.

In the trauma group, native thiol ( $p < 0.001$ ), total thiol ( $p < 0.001$ ), % dynamic disulfide/total thiol ( $p < 0.001$ ), albumin ( $p < 0.001$ ) and IMA ( $p < 0.001$ ) The decrease in the levels of 0.01 compared to the control group and the increase in dynamic disulfide ( $p = 0.001$ ) compared to the control group were statistically significant.

The cases in the trauma group were divided into two subgroups as surgery (n = 17) and follow-up (n = 84). This two groups' native thiol, total thiol, dynamic disulfide, disulfide / total thiol ratio, albumin and IMA levels were compared (see Table 3). No statistically significant difference was found between two subgroups in terms of their thiol-disulfide parameters.

The cases in the trauma group were also divided into two other sub-groups as multiple trauma (n = 54) and isolated organ trauma (n = 47). Two sub-groups were also similar in terms of their native thiol ( $p = 0.575$ ), total thiol ( $p = 0.794$ ), dynamic disulphide ( $p = 0.584$ ), disulfide / total thiol ratio ( $p = 0.414$ ), albumin ( $p = 0.792$ ) and IMA ( $p = 0.913$ ) levels (there was not table for this findings)

Lastly, the cases in the trauma group were divided into two sub-groups as penetrating (n = 15) and those who were exposed to blunt trauma (n = 86). Among two groups were not statistically different for native thiol ( $p = 0.65$ ), total thiol ( $p = 0.78$ ), dynamic disulfide ( $p = 0.41$ ), disulfide / total thiol ratio ( $p = 0.70$ ), albumin ( $p = 0.14$ ) and IMA ( $p = 0.98$ ) levels (see Table 4).

There was no difference between native thiol, total thiol, dynamic disulfide, disulfide / total thiol ratio, IMA, albumin levels and systems affected or organs (for all variables,  $p > 0.05$ ). There was also no difference between 2nd day of hospitalization and 5th day of hospitalization blood thiol-disulfide parameters tested with paired correlation test (for all,  $p > 0.05$ ).

#### 4. DISCUSSION

Free radicals stemming mainly from post-traumatic processes leads to damage in lipids, proteins in the cell membrane and DNA resulting in infections, multiple organ failure, acute failure of respiratory, renal and cerebral systems [30, 31]. Therefore, it is extremely important to know the levels of thiol to reduce free oxygen radicals, prevent harmful effects, and perform appropriate interventions to the patient after trauma. Oxidative stress is an indication of tissue damage from increased reactive oxygen species. Thiol inhibits cellular and tissue damage by inhibiting the effects of reactive oxygen species by lipid metabolism of the cell membrane.

It is known that oxidative stress increases after physical injuries in trauma patients. Although many studies have shown that reactive oxygen species in trauma increase and antioxidant levels to be decreased [30, 32, 33], there are two studies in the literature showing the relationship between trauma and dynamic thiol-disulfide balance [34, 35]. With this study, the relationship between trauma and thiol-disulfide and IMA levels in children was also shown.

In our study, total thiol, native thiol, disulfide/total thiol ratio were found to be significantly decreased significantly, as consistent with the literature and dynamic disulfide as increased in the trauma group compared to the control. IMA and albumin levels were also significantly decreased. In trauma group, however, there was not significant difference between surgery and follow-up sub-groups, or multiple and isolated trauma sub-groups, or type of trauma and systems affected or organs. These laboratory findings indicate that trauma results in a shift towards the oxidant side in favor of oxidative stress in children. However, there was no difference in trauma type, or whether there were surgery or not, multiple or isolated, systems or organs affected, and thiol/disulfide balance and IMA levels.

These results point out that thiol/disulfide balance and IMA levels cannot be used as an adjunctive marker in imaging and determining the affected system and organ from trauma during the follow-up period and deciding the surgical intervention. There was also not found statistical difference in total thiol, native thiol, dynamic disulfide, dynamic disulfide / total thiol rate, albumin or IMA levels between 2nd and 5th days of hospitalization of children with trauma. The development of a test that can help to determine the system and organ affected by the trauma more quickly and easily and can help in the decision of surgical intervention will help both early diagnosis and early surgical intervention.

#### 5. CONCLUSION

In conclusion, thiol-disulfide balance and IMA levels, in accordance with the literature, show changes in favor of oxidative stress in children with trauma, nonetheless, it is seen that it cannot be used as a laboratory marker that helps to show the system and organ affected by the trauma and to decide the surgical intervention.

**Conflicts of interest:** none.

**Ethical approval:** All procedures performed in studies involving human participants 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.

**Informed consent:** Informed consent was obtained from all individual participants included in the study

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**Table 1.** The frequency of the systems or organs that has been affected by trauma

Systems and organs affected	n
Spleen	37
Liver	34
Lungs	33
Extremities	17
Kidney	12
Central nervous system	10
Intestinal system	8
Pancreas	1
Bladder	1

n: frequency

**Table 2.** The comparison of laboratory findings of the variables in the groups

	Groups	n	Mean	Median	SD	Min	Max	p*
<b>Variables</b>								
<b>Native thiol (SH)</b>	Trauma	101	323.89	324.2	78.09	125.6	504.7	0.000
	Control	101	404.93	399.1	66.28	259.3	673.2	
<b>Total thiol (SH+SS)</b>	Trauma	101	368.45	369.1	81.84	150.5	565.2	0.000
	Control	101	444.18	369.68	72.81	279.6	742.5	
<b>Dynamic disulphide (SS)</b>	Trauma	101	22.22	21	7.97	4	52	0.001
	Control	101	19.64	19	4.8	10	35	
<b>Dynamic disulphide / Total thiol, %</b>	Trauma	101	0.06	0.05	0.024	0.01	0.18	0.000
	Control	101	0.044	0.043	0.008	0.03	0.08	
<b>Ischemia-modified Albumin (IMA)</b>	Trauma	101	0.49	0.47	0.22	0.14	1.74	0.000
	Control	101	0.63	0.65	0.17	0.27	1.32	
<b>Albumin</b>	Trauma	101	4.31	4.4	0.38	2.5	5	0.000

	Groups	n	Mean	Median	SD	Min	Max	p*
	Control	101	4.5	4.5	0.19	3.7	6	

n: frequency, SD: standard deviation, Min: Minimum, Max: Maximum, \*: Mann Whitney U test

**Table 3.** The comparison of the trauma sub-groups' laboratory findings

	Groups	n	Mean	SD	p*
<b>Variables</b>					
<b>Native thiol (SH)</b>	Surgery	17	317.1235	76.61708	0.61
	Follow-up	84	312.3574	64.22339	
<b>Total thiol (SH+SS)</b>	Surgery	17	4.4235	.23326	0.57
	Follow-up	84	4.2964	.38976	
<b>Dynamic disulphide (SS)</b>	Surgery	17	23.8824	10.01176	0.51
	Follow-up	84	21.6429	7.49882	
<b>Dynamic disulphide / Total thiol, %</b>	Surgery	17	.0631	.02397	0.73
	Follow-up	84	.0622	.02415	
<b>Ischemia-modified Albumin (IMA)</b>	Surgery	17	0.4622	0.1477	0.67
	Follow-up	84	0.5102	0.26865	
<b>Albumin</b>	Surgery	17	364.8588	77.71375	0.62
	Follow-up	84	355.7327	68.63171	

n: frequency, SD: standard deviation, \*: Mann Whitney U test

**Table 4.** The comparisons of the laboratory findings in terms of trauma type

	Groups	n	Mean	SD	p*
<b>Variables</b>					
<b>Native thiol (SH)</b>	Penetrating	15	322.79	55.16	0.65
	Blunt	86	311.48	67.94	
<b>Total thiol (SH+SS)</b>	Penetrating	15	364.78	61.33	0.78
	Blunt	86	355.96	71.55	
<b>Dynamic disulphide (SS)</b>	Penetrating	15	21.13	8.75	0.41
	Blunt	86	22.17	7.86	
<b>Dynamic disulphide / Total thiol, %</b>	Penetrating	15	0.06	0.01	0.70
	Blunt	86	0.06	0.03	
<b>Ischemia-modified Albumin (IMA)</b>	Penetrating	15	0.47	0.12	0.98
	Blunt	86	0.51	0.27	
<b>Albumin</b>	Penetrating	15	4.39	0.22	0.14
	Blunt	86	4.31	0.39	

n: frequency, SD: standard deviation, \*: Mann Whitney U test

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