



# Comparison of Pre-Operative and Post-Operative Thiol-Disulfide Levels in Acute Abdomen Patients

## Akut Karın Hastalarında Pre-operatif ve Post-operatif Tiyol-Disülfid Düzeylerinin Karşılaştırılması

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### Abstract

**Aim:** To evaluate role of thiol and disulfide homeostasis, a novel marker of oxidative stress, in the follow-up acute abdomen.

**Material and Method:** This prospective study included 107 patients (62 men and 45 women) with a diagnosis of acute abdomen (AA). In all patients, age, gender and cause of acute abdomen were recorded. In addition, native thiol (-SH), total thiol (tSH) and disulfide (-S-S-) levels at baseline, admission and on postoperative day 3 were prospectively recorded and -SS-/SH, -S-S-/tH and -SH/tSH ratios were calculated.

**Results:** When the causes of acute abdomen were assessed, it was seen that 72 patients (67.29%) underwent surgery due to appendicitis while 5 patients (4.67%) due to lower GIS perforation, 7 patients (6.54%) due to perforated peptic ulcer, 5 patients (4.67%) due to sigmoid volvulus, 4 patients (3.74%) due to strangulated hernia and 14 patients (13.08%) due to miscellaneous reasons. When thiol and disulfide levels were assessed as a single parameter, mean thiol level was  $316.71 \pm 78.16$  (327.5) at preoperative period and  $264.00 \pm 72.85$  (278.30) at postoperative period. The mean thiol level was significantly decreased at postoperative period ( $p < 0.001$ ). The disulfide/thiol ratio was  $5.17 \pm 1.56$  (5.16) at preoperative period and  $5.36 \pm 2.45$  (5.28) at postoperative period, indicating no significant difference ( $p = 0.563$ ).

**Conclusion:** In this study, it was found that monitoring these parameters resulting from thiol oxidation are valuable at both preoperative and postoperative period in patients with acute abdomen. Further studies are needed to optimize use of oxidative stress marker together with other established marker.

**Keywords:** acute abdomen, disulfide, laboratory study, thiol

### Öz

**Amaç:** Akut karın takibinde yeni bir oksidatif stres belirteci olan tiyol ve disülfid homeostazının rolünü araştırmak.

**Gereç ve Yöntem:** Bu çalışma prospektif olarak planlanmıştır. Bu çalışmaya akut karın(AK) tanılı 107 (62 erkek ve 45 kadın) hasta dahil edildi.107 hastanın yaşı, cinsiyeti, akut karın sebepleri, pre-operatif yatış anındaki ve post-operatif 3. gündeki native thiol (-SH), total thiol (tSH) ve disülfid (-S-S-) seviyeleri prospektif olarak kaydedilmiş ve -SS-/SH, -S-S-/tSH, -SH/tSH oranları hesaplanmıştır

**Bulgular:** Akut karına neden olan sebepler incelendiğinde 72 hasta (67,29%) apandisit, 5 hasta (4,67%) alt GIS perforasyonu, 7 hasta (6,54%) peptik ulcus perforasyonu, 5 hasta (4,67%) sigmoid volvulus, 4 hasta (3,74%) strangüle herni, 14 hasta ise (13,08%) diğer nedenlerden opere olmuştu. Hastaların tiyol ve disülfid seviyeleri tek parametre olarak incelendiğinde pre-operatif dönemde tiyol düzeyi ortalaması  $316,71 \pm 78,16$  (327,5) iken post-operatif dönemde ortalama  $264,00 \pm 72,85$  (278,30) olarak hesaplandı, istatistiki anlamlı farklılık gösterecek şekilde post-operatif dönemde azalma saptandı ( $p < 0,001$ ). Tiyol ve disülfid düzeylerinin birbirleri ile oranlarına bakıldığında pre-operatif dönemde disülfid/tiyol oranı ortalaması  $5,17 \pm 1,56$  (5,16) iken post-operatif dönemde ortalama  $5,36 \pm 2,45$  (5,28) olarak hesaplandı, istatistiki anlamlı farklılık gözlemlenmedi ( $p = 0,563$ ).

**Sonuç:** Bu çalışma AK'lı hastalarda tiyol oksidasyonunun bir sonucu olarak ortaya çıkan bu parametrelerin takibinin, gerek preoperatif ve gerekse postoperatif dönemde önem arz ettiği bulundu. Bu yeni oksidatif stres belirtecinin diğer yerleşik yaklaşımlarla birlikte kullanımını optimize etmek için daha ileri çalışmalar gereklidir.

**Anahtar Kelimeler:** akut karın, disülfid, laboratuvar çalışması, tiyol



## INTRODUCTION

Acute abdomen can occur due to an infection, inflammation, vascular occlusion or intestinal obstruction. The patients generally present with sudden onset of abdominal pain with nausea or vomiting.<sup>[1]</sup>

History and physical examination are important in the approach to the patient with acute abdomen. The localization and character of the pain are key elements in the diagnosis. The presence of free air in the abdomen can be presented with pain in all quadrants. Bowel sounds are assessed during auscultation. Auscultation can reveal lacking or decreased bowel sounds while palpation can show rebound tenderness suggesting peritonitis. The causes of acute abdomen include appendicitis, perforated peptic ulcer, acute pancreatitis, perforated sigmoid diverticulitis, ovarian torsion, volvulus, ruptured aortic aneurysm, splenic or hepatic rupture and intestinal ischemia.<sup>[2,3]</sup>

Despite advances in the diagnosis and treatment of acute abdomen, laboratory parameters used in the follow-up remain to be routine blood parameters. In recent years, several studies have demonstrated potential role of oxidative stress parameters resulting from acute abdomen in the pathogenesis of the disease.<sup>[4-6]</sup> In fact, it was proven that some biochemical parameters used to define oxidative stress and inflammation are markers for diagnosis and identification of clinical aspects in acute appendicitis, one of the leading causes of acute abdomen. It is known that plasma thiols are free radical scavengers and have antioxidant function through several mechanisms. Again, it is also known that plasma total thiol measurement and estimation of thiol/disulfide homeostasis are representative for excessive free radical formation in several diseases.<sup>[5,6]</sup>

In this study, we investigated clinical value of serum thiol and disulfide levels in patients with acute abdomen.

## MATERIAL AND METHOD

The study was approved by Ethics Committee on Clinical Research of Health Sciences University, Ankara Keçiören Teaching and Research Hospital (28.12.2016 / 2012-KAEK-15/1245).

In the study, we screened patients who were examined and underwent surgery with a diagnosis of acute abdomen at General Surgery Clinic of Health Sciences University, Ankara Keçiören Teaching and Research Hospital. The patients aged <18 years; those with history of smoking or alcohol consumption; those with presence of any infection foci other than acute abdomen; those with acute or chronic renal disease; those with known hematological-oncologic disease; those with heart disease, chronic obstructive pulmonary disease, diabetes mellitus, hypertension or rheumatic disease; and those using any

drug with antioxidant effect were excluded. Overall, 107 patients were included to the study. The plasma samples were obtained from patients and stored at -80°C until assays in central laboratory. Thiol-disulfide homeostasis primarily involves degradation of dynamic disulfide bonds (-S-S-). The degradation into functional thiol groups (-SH) was achieved by sodium borohydride. Total thiol content was analyzed using modified Ellman's reagent. The sodium borohydride residues were removed by formaldehyde during process. The amount of native thiol (-SH) was estimated from total thiol (tSH) content. The half of the difference was defined as amount of -S-S- bound. In all patients, age, gender and cause of acute abdomen were recorded. In addition, native thiol (-SH), total thiol (tSH) and disulfide (-S-S-) levels at baseline, admission and on postoperative day 3 were prospectively recorded and -SS-/-SH, -S-S-/tSH and -SH/tSH ratios were calculated.

All statistical analyses were performed using IBM SPSS for Windows version 26.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics are presented as count and percent for categorical variables whereas mean  $\pm$  standard deviation and median for numerical variables. The normality of data distribution was assessed using Shapiro-Wilk test. The relations among variables were analyzed using Pearson's correlation tests and Spearman's correlation coefficient where appropriate. For binary comparisons, Paired samples t test was used to compare -S-S- level while Wilcoxon signed rank test was used to compare SH and tSH levels and -S-S-/-SH, -S-S-/tSH and -SH/tSH ratios. A p value <0.05 was considered as statistically significant.

## RESULTS

Mean age was 42.78 $\pm$ 18.3 (38) years in the study population. The study included 62 men (57.9+%) and 45 (42.06%) women. When the causes of acute abdomen were assessed, it was seen that 72 patients (67.29%) underwent surgery due to appendicitis while 5 patients (4.67%) due to lower GIS perforation, 7 patients (6.54%) due to perforated peptic ulcer, 5 patients (4.67%) due to sigmoid volvulus, 4 patients (3.74%) due to strangulated hernia and 14 patients (13.08%) due to miscellaneous reasons (**Table 1**).

When thiol and disulfide levels were assessed as a single parameter, mean thiol level was 316.71 $\pm$ 78.16 (327.5) at preoperative period and 264.00 $\pm$ 72.85 (278.30) at postoperative period. The mean thiol level was significantly decreased at postoperative period (p<0.001). Mean total thiol level was 348.6 $\pm$ 83.55 (361.2) at preoperative period and 290.98 $\pm$ 77.39 (309.70) at postoperative period. There was a significant decrease in total thiol level at postoperative period (p<0.001). Mean disulfide level was 15.95 $\pm$ 5.02 (16.15) at preoperative period and 13.49 $\pm$ 5.43 (13.85) at postoperative period. Again, disulfide level was significantly decreased at postoperative period (p<0.001).

**Table-1: Comparison of thiol-disulfide levels between preoperative and on postoperative day 3**

Variables	Pre-operative	Post-operative	Statistical Significance
Age		42.78±18.3 (38)	
Gender			
Male		62 (57.94%)	
Female		45 (42.06%)	
Thiol (-SH)	316.71±78.16 (327.5)	264.00±72.85 (278.30)	<0.001
Total thiol (tSH)	348.6±83.55 (361.2)	290.98±77.39 (309.70)	<0.001
Disulfide (-S-S-)	15.95±5.02 (16.15)	13.49±5.43 (13.85)	<0.001
-S-S-/-SH	5.17±1.56 (5.16)	5.36±2.45 (5.28)	0.563
-S-S-/tSH	4.65±1.28 (4.68)	4.75±1.94 (4.78)	0.584
-SH/tSH	90.7±2.55 (90.64)	90.49±3.87 (90.45)	0.584
Diagnosis			
Appendicitis		72 (67.29%)	
Peptic ulcer perforation		7 (6.54%)	
Lower GIS Perforation		5 (4.67%)	
Sigmoid volvulus		5 (4.67%)	
Strangulated Hernia		4 (3.74%)	
Other		14 (13.08%)	

When native thiol to disulfide ratio was assessed, it was found that disulfide/native thiol ratio was  $5.17\pm 1.56$  (5.16) at preoperative period and  $5.36\pm 2.45$  (5.28) at postoperative period, indicating no significant difference ( $p=0.563$ ). Mean disulfide/total thiol level ratio was  $4.65\pm 1.28$  (4.68) at preoperative period and  $4.75\pm 1.94$  (4.78) at postoperative period, indicating no significant difference ( $p=0.584$ ). Again, mean native thiol/total thiol ratio was  $90.7\pm 2.55$  (90.64) at preoperative period and  $90.49\pm 3.87$  (90.45) at postoperative period, indicating no significant difference ( $p=0.584$ ).

## DISCUSSION

We showed that thiol and disulfide parameters are valuable tests in the diagnosis and differential diagnosis of acute abdomen. These parameters were investigated in the diagnosis of many causes of acute abdomen.[7-9] In addition to inflammatory markers, oxidative stress markers have recently become focus of interest in research efforts. In the literature, there is limited number of experimental and clinical trials addressing relationship between acute abdomen and oxidative stress.<sup>[10,11]</sup>

Chemically, thiols are formed by carbon atom binding to a sulfur atom. In addition, the carbon also binds a hydrogen atom. This structure also includes a sulfhydryl group.<sup>[12]</sup> The disulfide bonds can be reduced to thiol group. Thiols comprise majority of total antioxidant capacity, providing protection against reactive oxygen species. In addition, they play role in detoxification and programmed cell death.<sup>[13,14]</sup>

Recently, it was shown that disruption in thiol/disulfide homeostasis plays role in the pathogenesis of several acute and chronic diseases.<sup>[15]</sup> The measurement of serum thiol level reveals their roles in antioxidant defense system.<sup>[14]</sup> Thus, the decreased amounts at postoperative period can be considered as a marker for simultaneous reduction in

oxidative stress. Dynamic thiol/disulfide measurement was first introduced by Ere and Neselioglu.<sup>[13]</sup> In our study, we also investigate dynamic thiol/disulfide homeostasis parameters and their value in the diagnosis in patients with acute abdomen.

In clinical practice, C-reactive protein and white blood cell (WBC) count are used to predict severity of inflammation at preoperative period and during follow-up at postoperative period in patients with acute abdomen. Previous studies have shown that CRP value is increased in case of acute abdomen and reaches to maximum level in case of perforation. Again, it was shown that the serum CRP level increased at preoperative period was decreased at postoperative period.<sup>[16-19]</sup> Similarly, it was found that serum thiol and disulfide levels were significantly increased at preoperative period, which were, then, decreased at postoperative period. Given the correlation between severity of inflammation and elevation in disulfide/native thiol ratio, it can be suggested that disulfide/native thiol ratio can be used as a marker for disease progression and activity. Although plasma thiol and disulfide measurements aren't routinely used in the diagnosis of acute abdomen, the serum levels with apparent increase at baseline are important in the diagnosis while decreased serum levels at postoperative period can be considered as a marker for regression of disease and recovery.

This study has some limitations, Firstly, it is a pilot study addressing thiol and disulfide homeostasis parameters in the diagnosis and follow-up of acute abdomen for the first time. Secondly, the sample size is relatively limited in this single-center study. Thirdly, we failed to correlate the diagnostic tools such as serum procalcitonin, CRP, WBC count, sonography and/or computed tomography with parameters of thiol and disulfide homeostasis. Finally, thiol/disulfide homeostasis was studied in frozen samples, rather than fresh samples, at a central laboratory. However, the prospective

design is an important strength of the study. In addition, our results showed that these parameters can be used in the diagnosis and follow-up of patients with acute abdomen in the future. Moreover, our study is valuable as it offers novel parameters that can be considered in case of inconclusive clinical and laboratory findings.

## CONCLUSION

In this study, it was shown that the serum thiol and disulfide levels were increased at preoperative period while they were decreased at postoperative period in patients with acute abdomen but their ratio to each other showed no significant change. Thus, above-mentioned parameters produced by thiol oxidation are valuable in the diagnosis at preoperative period and as a marker for recovery at postoperative follow-up. Further studies are needed to optimize use this novel oxidative marker together with established parameters.

## ETHICAL DECLARATIONS

**Ethics Committee Approval:** The study was carried out with the permission of Kecioren Training and Research Hospital Ethics Committee (Date: 28.12.2016, Decision No: 2012-KAEK-15/1245 ).

**Informed Consent:** All patients signed the free and informed consent form.

**Referee Evaluation Process:** Externally peer-reviewed.

**Conflict of Interest Statement:** The authors have no conflicts of interest to declare.

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**Author Contributions:** All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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