Investigation of thiol/disulfide homeostasis changes and their relationship with prognosis in sepsis patients

Sepsis hastalarında tiyol/disülfid homeostaz değişikliklerinin ve prognoz ile ilişkisinin araştırılması

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Abstract

Aim: Sepsis is the most common life-threatening syndrome. Oxidative stress is one of the mechanisms involved in the pathogenesis of sepsis. Thiols are antioxidant buffers. Changes in thiol parameters refer to changes in oxidative balance. In this study, we aimed to investigate thiol homeostasis changes in sepsis patients.

Methods: We included 99 patients (53 patients diagnosed with sepsis in the Intensive Care Unit and 46 controls). Sepsis patients were divided into two groups based on their diagnosis, follow-up, and disease severity: sepsis and septic shock. Total thiol and native thiol levels were analyzed in the patients and controls. Disulfide levels were calculated. The thiol parameters were compared between the patient and control groups, sepsis and septic shock, mortality, and survivor groups.

Results: In sepsis patients, the disulfide/native thiol and disulfide/total thiol rates were significantly higher (p <0.001; <0.001), and the native thiol and total thiol levels were lower (p <0.001; <0.001) than controls. Compared to the sepsis group, the disulfide level was higher p:0.001, and native thiol and total thiol levels were lower (p <0.001; 0.009) in the septic shock patients. The results from patients who survived showed no statistical difference from those patients who died.

Conclusion: The differences in thiol homeostasis parameters showed that sepsis patients had higher oxidative stress compared to the controls. The oxidative stress changes were parallel to the disease severity. The septic shock patients had higher oxidative stress compared to the sepsis patients. Diagnosis is still a problem despite the many new biomarkers for sepsis. Analyzing thiol parameters and assessing them will contribute to the diagnosis and follow-up of sepsis patients.

Keywords: Oxidative stress; prognosis; septic shock; sepsis

Öz

Amaç: Sepsis hayatı tehdit eden en yaygın sendromdur. Oksidatif stres sepsis patogenezinde rol oynayan mekanizmalardan biridir. Tiyoller antioksidan tamponlardır. Tiyol parametrelerindeki değişiklikler, oksidatif dengedeki değişiklikleri ifade eder. Bu çalışmada sepsis hastalarında tiyol homeostazındaki değişiklikleri araştırmayı amaçladık.

Yöntemler: Bu çalışmaya 99 hasta dâhil edildi: 53 hasta (Yoğun Bakım Ünitesi'inde sepsis tanısı konulan) ve 46 kontrol. Sepsis hastaları tanı, takip ve hastalık şiddetlerine göre iki gruba ayrıldı: sepsis ve septik şok. Hasta ve kontrollerde total tiyol ve native tiyol seviyeleri ölçüldü. Disülfit seviyeleri hesaplandı. Tiyol parametreleri hasta ve kontrol grupları, sepsis ve septik şok, ölen ve hayatta kalan gruplar arasında karşılaştırıldı.

Bulgular: Sepsis hastalarında kontrollere göre disülfit/native tiyol ve disülfit/total tiyol oranları belirgin daha yüksek (p: <0.001, p: <0.001), native tiyol ve total tiyol seviyeleri daha düşüktü (p: <0.001, p: 0.00). Sepsis grubuyla karşılaştırıldığında septik şok hastalarında disülfit düzeyi daha yüksek p: <0.001, native tiyol ve total tiyol düzeyleri daha düşüktü (p: <0.001, p: 0.009). Hayatta kalan hastalardan alınan sonuçlar, ölen hastalardan istatistiksel olarak farklılık göstermedi.

Sonuç: Tiyol homeostaz parametrelerindeki farklılıklar, sepsis hastalarının kontrollere kıyasla daha yüksek oksidatif strese sahip olduğunu gösterdi. Oksidatif stres değişiklikleri hastalık şiddeti ile paraleldi. Sepsis hastalarına kıyasla septik şok hastalarında oksidatif stres daha yüksekti. Sepsiste birçok yeni biyobelirteç olmasına rağmen tanı hala bir sorundur. Tiyol parametrelerinin ölçülmesi ve değerlendirilmesi sepsis tanı ve takibine katkı sağlayacaktır.

Anahtar Sözcükler: Oksidatif stress; prognoz; septik şok; sepsis

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INTRODUCTION

Sepsis is a life-threatening syndrome causing organ dysfunction in response to infections. It is one of the most common reasons for intensive care unit (ICU) admission and mortality (1,2). The prevalence of both community- and healthcare-associated sepsis is rising, particularly among the elderly (1,2). Recognition and early treatment are the main factors for survival. There are improvements in sepsis and mortality. Despite the use of inflammatory biomarkers, there are still some issues with early diagnosis and treatment (1,3,4). Oxidative stress parameters are new biomarkers in sepsis diagnosis (3,5).

Increased oxidative stress is expected in sepsis patients as a result of pro-oxidant and antioxidant inadequacy (6-10). According to recent research data, the level of increased oxygen species can predict mortality (8,9). Thiol-disulfide homeostasis is one of the mechanisms that can measure oxidative stress. Disulfide and thiols are in a changing balance in plasma during the process of eliminating free radicals (11).

In this study, we aimed to investigate thiol homeostasis parameter changes in sepsis patients and the use of thiol homeostasis in the diagnosis and follow-up of sepsis. We think that our results will contribute to this issue, for which there is still no ideal biomarker.

MATERIAL AND METHODS

During the time of the study, patients who were admitted to the ICU with a diagnosis of sepsis and who were diagnosed with sepsis during follow-up in the ICU were included in this study. For sepsis diagnosis, the current sepsis criteria were used (12). The patients who met two or more quick Sequential Organ Failure Assessment (SOFA) criteria or had an increase of more than 2 points in their SOFA scores were diagnosed with sepsis. Among the sepsis patients, those with persistent hypotension despite vasopressor treatment and lactate levels >2 mmol/L were diagnosed with septic shock (12).

The power analysis before data collection showed that for 95% power, a 0.83 effect size, and a 0.05 significance level, the study required 78 patients, 39 for each group. The retrospective power analysis showed that the study required 36 patients (18 patients for each group) with 95% power, a 1.23 effect size, and a 0.05 significance level. A total of 53 patients with sepsis in the ICU and 46 controls were included in the study.

All patients were checked for signs and symptoms of sepsis every day in the ICU. Patients with a confirmed diagnosis of sepsis were included in the study. Patients who did not meet the diagnostic criteria were excluded from the study. Blood samples were taken from all of the patients diagnosed with sepsis within one hour to analyze thiol tests. The patients with late or no blood samples were excluded from the study. According to their clinical status, patients were separated into groups with sepsis and septic shock based on their clinical and laboratory findings. All patients in the study were monitored for prognosis and mortality. Patients who died within seven days and patients who survived were investigated.

The control group consisted of people who went to the outpatient department of the hospital for routine health checks and did not have any acute health problems. No patient with any symptoms or acute diagnosis was included in the control group. Written informed consent was obtained from all of the patients.

Before the analysis, all the samples from the patients and controls were stored at -80 °C. All tests were analyzed at the same time at the end of the study period. Total thiol (SH+SS) and native thiol (SH) levels were analyzed with a clinical chemistry analyzer (Roche, Cobas 501, Manheim, Germany). Disulfide results, disulfide (SS) and native thiol ratios, and disulfide and total thiol ratios were determined (11). All of the test results were examined, and the results with a hemolytic blood sample were excluded from the study.

Statistical Analysis

Statistical Package for the Social Sciences software for Windows, version 25.0 (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. The demographic features of the patients were analyzed using descriptive statistical analysis. Normality was checked using the Kolmogorov-Smirnov test. In all thiol parameters, patients and controls, sepsis and septic shock patients, and survival and mortality groups were compared. The independent sample t-test was used to compare groups in normally distributed groups. For non-normally distributed data, the Mann-Whitney U test was used to

compare the groups. A p-value ≤ 0.05 is considered significant.

This study was approved by the Clinical Research Ethics Committee of YBÜ Yenimahalle Research and Training Hospital (date:12.14.2015 , decision no: 2015/47).

RESULTS

In the study, 99 people were included: 53 patients and 46 controls. The eight patients whose blood samples weren't taken on time and seven patients with hemolyzed blood samples were excluded from the study (Figure 1). A total of 37 sepsis and 46 control patients were in the study. The mean age of the patients was 75.6 (53-97±10,9). 17 (45.9%) of them were male. The control group's mean age was 71,7 (52-9310,1).21 (45.6%) of them were male (Table 1). The most common sources of infections were 25 (67.5%) pneumonia and 6 (16.2%) urinary tract infections. Among the patients, 23 (62%) were diagnosed with sepsis, and fourteen were in septic shock. The mortality rate was 12 (32.4%) in the first seven days.

Except for the disulfide level, there were statistically significant differences between the patients and the controls in native thiol, total thiol levels, disulfide/native thiol, disulfide/total thiol, and native thiol/total thiol ratios. In the patient group, disulfide/total thiol and disulfide/native thiol rates were significantly higher (p: <0.001, p: <0.001) and total thiol and native thiol levels were lower (p: <0.001, p: <0.001) than in the control group (Table 2).

The sepsis and septic shock patients had statistically significant differences in thiol parameters. Disulfide levels, disulfide/native thiol, and disulfide/total thiol ratios were significantly higher in septic shock patients compared to sepsis patients (p: 0.001, p: <0.001, p: <0.001) (Table 3).

Thiol homeostasis parameters did not show a statistically significant difference between patients who died within seven days and those who survived (Table 4).

DISCUSSION AND CONCLUSION

Thiols are antioxidant buffers for many oxidants. Dynamic thiol-disulfide homeostasis is an antioxidant defense mechanism (11). An increase in disulfide/native thiol, disulfide/total thiol, and a decrease in native thiol/total thiol are signs of the deterioration of oxidative balance. A decrease in thiol levels indicates a

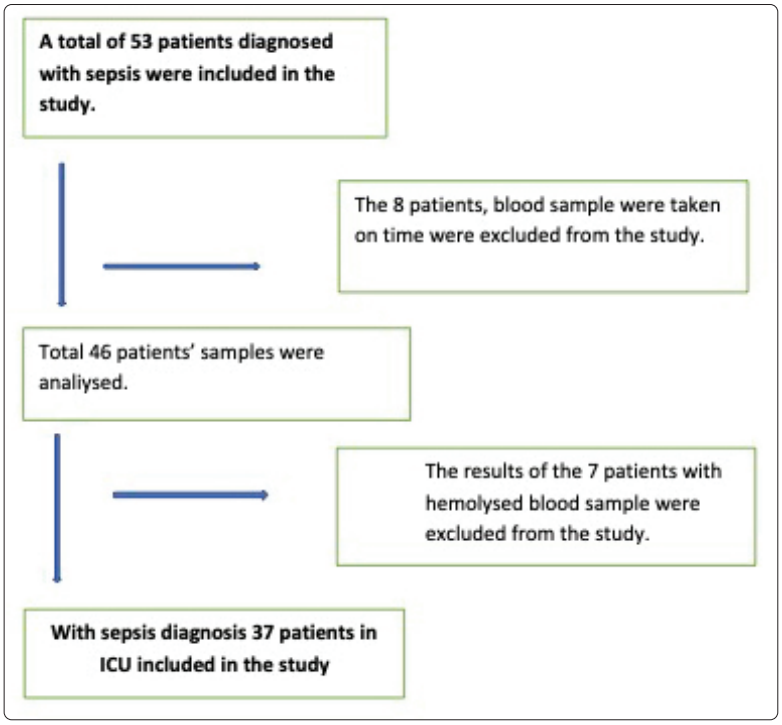

Figure 1: The sepsis patients were included in the study

Table 1. Demographic features of the patient and control groups

	Patient	Control	*p*
Female/male, n	20/ 17	25/21	0.900
Total (mean age, %)	37 (75,6)	46 (71.7)	0.600
Min-Max	53-97	52-93	

Max: Maximum, Min: Minimum, n: Number, %: Percent

Table 2. Thiol homeostasis results in patient and control groups

	Patient (n=37)	Control (n=46)	
	Median (Min-Max)	Median (Min-Max)	*p*
Native Thiol	89.40 (10.7-363.2)	375.7 (190.3-568.5)	<0.001
Total thiol	111.90 (24.8-416.5)	418.7 (204.1-591.9)	<0.001
Disulfide	17.5 (1.0-83.0)	27.6 (2.4-79.8)	0.160
Disulfide/native thiol	23.9 (0.5-475.2	6.7 (0.4-22.7)	<0.001
Disulfide/total thiol	19.3 (0.5-82.6)	6.3 (0.4-18.5)	<0.001
Native thiol/ total thiol	80.7 (17.3-99.4)	93.6 (81.4-99.5)	<0.001

Max: Maximum, Min: Minimum, n: Number, %: Percent

Table 3. Thiol parameters in sepsis and septic shock patients

	Sepsis (n=23)	Septic shock (n=14)	
	Median (Min-Max)	Median (Min-Max)	*p*
Native Thiol	132.5 (30.4-363.2)	67.4 (10,7-153,9)	<0.001
Total thiol	142.4 (40.4-416.5)	94.0 (24.8-173.8)	0.009
Disulfide	14.3(1.0-53.3)	30.2 (14.1-83.0)	0.001
Disulfide/native thiol	12.0 (0.5-55.1)	50.3 (12.9-475.2)	<0.001
Disulfide/total thiol	10.7(0.5-35.5)	33.4(11.4-82.6)	<0.001
Native thiol/ total thiol	89.2(64.4-99.4)	66.5(17.3 88.5)	<0.001

Max: Maximum, Min: Minimum, n: Number, %: Percent

Table 4. The thiol results of the patients who died in 7 days and survived

	Died in 7 days. (n=12)	Suvived (n=25)	*p*
	Median (Min-Max)	Median (Min-Max)	
Native Thiol	70.5 (10.2-238.6)	97.4 (10.9-363.2)	0.290
Total thiol	93.5 (24.8-252.9)	132.8 (40.4-416.5)	0.150
Disulfide	15.0 (1.0-32.7)	19.9 (7.6-83.0)	0.280
Disulfide/native thiol	32.8 (0.5-131.7)	21.7 (4.3-475.2)	0.830
Disulfide/total thiol	24.3 (0.5-56.8)	17.8 (4.1-82.6)	0.830
Native thiol/ total thiol	75.6 (43.1-99.4)	82.1 (17.3-95.8)	0.830

Max: Maximum, Min: Minimum, n: Number, %: Percent

weakened antioxidant defense mechanism (11). Thiol homeostasis has been studied in patients with diabetes, cancer, liver and kidney disorders, and hyperemesis gravidarum (13-15).

In some studies, an increase in oxidative stress, a significant decrease in antioxidants, and an increase in the oxidized form were observed (7,8,16-19). Our test results were as expected in the patient and control groups (11). The patients had lower total and native thiol levels compared to the control group. Contrary to increased oxidative stress, the disulfide result was higher in the control group; on the other hand, the disulfide/total thiol, disulfide/native thiol, and native thiol/total thiol rates were as expected. In the study by Ayar et al. on pediatric sepsis patients, there was a disulfide/total thiol rate discrepancy with oxidative stress (16). The difference observed in disulfide level in our study was evaluated in connection with the dynamic character of thiol homeostasis and ongoing changes in parameters, as in pediatric sepsis patients (16). Since thiol homeostasis is a dynamic process, it is necessary to monitor all parameters together rather than a single parameter to decide on increased oxidative stress. The changes in disulfide/total thiol, disulfide/native thiol, and native thiol/total thiol indicate changes in oxidative balance. In both studies, the thiol homeostasis balance was altered in the direction of oxidative stress in sepsis patients.

The thiol homeostasis parameters were significantly different in the sepsis and septic shock patients. As a more severe form of the disease, septic shock patients had higher oxidative stress than sepsis patients. In pediatric sepsis patients, thiol homeostasis and disease severity were not correlated (16). On the other hand, other studies like ours found significant correlations between antioxidant levels, oxidative stress, and disease severity. They proposed using these parameters as prognostic biomarkers among sepsis patients (9,18,19).

In some studies, sepsis patients who didn't have enough antioxidants were more likely to die (8,9,18,20-22). In our study, thiol parameters weren't statistically different in the survivors compared to those who died. There wasn't any statistically significant difference between survivors and non-survivors among pediatric sepsis patients (16). Although the difference wasn't statistically significant, the changes in thiol homeostasis indicated higher oxidative stress in dying patients than in survivors.

The limitation of our study is that not performing serial measurements of thiol parameters alongside an assessment of the patient's clinical situation would have provided clearer information about the prognosis and thiol homeostasis relationship.

In conclusion, thiol homeostasis parameters showed a significant oxidative stress increase in the sepsis group compared to the septic shock group and in the patient group compared to the control group. The difference in thiol parameters between the mortality groups wasn't statistically significant. We think that this is because thiol homeostasis parameters were not evaluated serially and were not associated with the current clinical situation, which is the limitation of our study. Some parameters weren't as expected in thiols. It was a result of the dynamic thiol homeostasis process. To assess changes in thiol homeostasis in response to changes in clinical conditions, all parameters together need to be evaluated.

Conflict-of-Interest and Financial Disclosure

The authors declare that they have no conflict of interest to disclose. The authors also declare that they did not receive any financial support for the study.

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