RESEARCH ARTICLE

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Thiol-Disulphide Homoeostasis in COVID-19: Evaluation of its Relationship with Complete Blood Count Parameters

ABSTRACT

Objective: In this study, we aimed to evaluate the relationship between thiol-disulfide homoeostasis and hemogram parameters in COVID-19 patients.

Methods: Total thiol(TT), Native thiol(NT), dynamic disulfide status(DDS), DDS/NT, DDS/TT, NT/TT ratio and CBC parameters were analyzed in 68 patients with positive COVID-19 and 31 healthy individuals.

Results: TT, NT, DD, hemoglobin and hematocrit levels were higher in the control group than in patient groups. TT, NT, DD and lymphocyte levels of COVID-19 patients treated in medical floor were higher than those treated in intensive care unit; WBC, neutrophil and NLR were low($P<0.05$).PLR was higher in intensive care patients compared with the control group($P<0.05$).COVID-19 patients who did not need mechanical ventilation were retrospectively evaluated according to their mortality. TT, NT, DDS and lymphocyte levels were higher; WBC, Neutrophil, PLR and NLR were lower($P<0.05$) in survivors. The diagnostic performance of TT, NT and DDS levels to define requirement of intensive care treatment in COVID-19 patients were evaluated by using Receiver Operating Characteristic (ROC) curve analysis. By using ROC analysis, the optimum cut-off points for of TT, NT and DDS levels showed high sensitivity and specificity for requirement of intensive care treatment($P<0.05$).

Conclusions: According to our results, it has been observed that the thiol-disulfide balance is disrupted In COVID-19 patients. It may be beneficial to monitor the thiol-disulfide balance in the follow-up and treatment of the patients.

Keywords: COVID-19, Total Thiol, Native Thiol, Dynamic Disulfide Status, Complete Blood Count.

COVID-19'da Tiyol-Disülfid Dengesi: Tam Kan Sayımı Parametreleri ile İlişkisinin Değerlendirilmesi

ÖZET

Amaç: Bu çalışmada, COVID-19 hastalarında tiyol-disülfid homoeostazı ile hemogram parametreleri arasındaki ilişkiyi değerlendirmeyi amaçladık.

Gereç ve Yöntem: Total tiyol (TT), Native tiyol (NT), dinamik disülfid durumu (DDS), DDS / NT, DDS / TT, NT / TT oranı ve CBC parametreleri COVID-19 pozitif 68 hasta ve 31 sağlıklı bireyde analiz edildi.

Bulgular: Kontrol grubunda TT, NT, DD, hemoglobin ve hematokrit düzeyleri hasta gruplarına göre daha yüksekti. Serviste tedavi gören COVID-19 hastalarının TT, NT, DD ve lenfosit seviyeleri yoğun bakım ünitesinde tedavi edilenlere göre daha yüksekti; WBC, nötrofil ve NLO düşüktü ($P <0.05$). Yoğun bakım hastalarında PLR, kontrol grubuna göre daha yüksekti ($P <0.05$). Mekanik ventilasyona ihtiyaç duymayan COVID-19 hastaları mortalitelerine göre geriye dönük olarak değerlendirildi. TT, NT, DDS ve lenfosit seviyeleri daha yüksekti; Hayatta kalanlarda WBC, Nötrofil, PLR ve NLR daha düşüktü ($P <0.05$). COVID-19 hastalarında yoğun bakım tedavisi gereksinimini tanımlamak için TT, NT ve DDS düzeylerinin tanısal performansı, ROC eğrisi analizi kullanılarak değerlendirildi. ROC analizine göre, yoğun bakım tedavisi gereksinimi için TT, NT ve DDS düzeyleri optimum kestirim değerlerinde, yüksek duyarlılık ve özgüllük göstermiştir ($P <0.05$).

Sonuç: Sonuçlarımıza göre COVID-19 hastalarında tiyol-disülfid dengesinin bozulduğu görüldü. Hastaların takip ve tedavisinde tiyol-disülfid dengesinin izlenmesi faydalı olabilir.

Anahtar Kelimeler: COVID-19, Toplam Tiyol, Doğal Tiyol, Dinamik Disülfür Durumu, Tam Kan Sayımı.

INTRODUCTION

The virus named SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) caused Coronavirus disease (COVID-19), which started in the city of Wuhan in December 2019 and spread rapidly to the World (1). This disease was declared as a pandemic by the World Health Organization (WHO) on March, 2020 (2) Coronaviruses, including SARS-CoV-2 are enveloped RNA viruses that can cause respiratory, intestinal, liver and neurological diseases in human, other mammals, and birds (3). It can cause symptoms such as fever, cough, dyspnea, and myalgia. Shock, acute respiratory distress syndrome (ARDS), acute heart damage and acute kidney injury may develop and progress to death. In addition to radiological findings, parameters such as complete blood count (CBC), C-reactive protein (CRP) and D-Dimer are used in the diagnosis and follow-up of the disease (4).

It has been known that oxidative stress has important role in the course of viral infections. It also plays an important role in the proper functioning of the immune system and host defense against pathogens (5). Reactive oxygen species (ROS) are produced in phagocytes to destroy pathogenic macromolecules directly. It can also take place indirect antimicrobial processes (5, 6).

It is known that oxidoreductases associated with the cell surface play a role in the entry of viruses into host cells. For entry of enveloped viruses into target cells, interaction between viral envelope glycoproteins and cellular receptors occur on the surface of the target cell. Conformational changes are produced in the receptors as a result of Thiol / disulfide exchange reactions occurring in glycoproteins. Finally, viral particles enter into host cell with clathrin-mediated or clathrin-independent endocytosis (7, 8). Increased ROS production due to viral infection trigger pro-inflammatory response by affecting several transcription factors such as NF-κB (9). Although cells have special antioxidant systems to deal with increased ROS production, these systems are rapidly depleted during viral infection and uncontrolled oxidative stress occurs. Prolonged oxidative stress can then cause apoptosis or necrosis, leading to a decrease in lymphocyte cell numbers (6, 10).

Thiols are most important defense system against reactive species due to sulfhydryl groups (SH) in their structure. SH groups can be oxidized by the oxidant molecules in the environment and converted into reversible disulfide (SS) bond structures (11). The disulfide bond structures formed in this way are reduced back to thiol (SH) groups, and the thiol-disulfide balance is preserved (12). This balance has important roles in antioxidant protection, detoxification, apoptosis, regulation of enzymatic activity, and cellular signaling mechanisms. Therefore, evaluation of Thiol-disulfide balance in patients in COVID-19 infection may reveal some new information about this disease (12, 13).

In this study, we aimed to evaluate the oxidative stress level in COVID-19 patients with Thiol-disulfide balance as a new oxidative stress marker. We also searched its relationship with underling chronic diseases, therapeutic drugs used in treatment, clinical course, lymphopenia, leukocytosis level, neutrophil lymphocyte ratio (NLR) and platalet lymphocyte ratio (PLR) in COVID-19 patients.

MATERIAL AND METHODS

This study was conducted with 68 individuals who applied to XX University Medical Faculty Training and Research Hospital between May 15 and August 15 with positive COVID-19 PCR results, which were treated and followed up in the service and intensive care unit (ICU), as well as any chronic diseases and drugs admitted to the general internal medicine outpatient clinic on the same dates as a control group with negative PCR test results. Individuals with positive COVID-19 PCR results were divided into two groups as inpatients (33) and ICU (35) patients. Within the scope of the study, the patients' age, gender, chronic diseases and clinical information (application complaints, hospitalization in the medical floor-ICU, intubation status, death or discharge status and the drugs they used) were obtained through the hospital automation system. Patients who refused to participate in the study and were under 18 years of age were excluded from the study. Remaining parts of the samples taken during routine analysis were kept under appropriate conditions and no additonal sample was taken.

After the samples arrived at the biochemistry laboratory, CBC parameters were analyzed immediately. Venous blood samples were centrifuged at 1500 g for 10 minutes after the coagulation process was completed. The samples were not hemolyzed and lipaemic. Sera for total thiol and native thiol measurement were stored at -80 oC until the analyzed. All samples were allowed to come to room temperature and were carefully mixed to homogenize.

Serum Total Thiol, Native Thiold levels were measured in the Olympus AU5800 (BeckmanCoulter, Inc. Brea, CA92821 USA) autoanalyzer using the spectrophotometric method developed by Erel and Neselioğlu (14) CBC were measured by laser measurement and LED Flow Cell method on a CELL-DYN 3700 CD-3700SL (AbbottDiagnostics Liquid, Abbott Laboratories Abbott Park IL, 60064, USA) device. Dynamic disulfide status (DDS) was calculated by taking half of the difference between the measured total thiol (TT) and Native thiol (NT) levels. DDS / NT, DDS / TT, NT / TT were calculated.

All values obtained were evaluated in the SPSS (ver. 20.0; SPSS, USA) program. The mean,

median, min-max value and standard deviations of the measurement results were calculated. The Shapiro-Wilk Test was used to determine whether the data conformed to normal distribution. Student-t test was used when parametric test conditions were met in groups with two independent variables, Mann-Whitney U test if not provided, One-Way Analysis of Variance if parametric test conditions were met in groups with more than two independent variables, and Kruskal-Wallis test if not. Pearson Chi-Square test was used for categorical variables. Correlation and analysis were performed to evaluate the relationship between thiol-disulfide levels of patients with CBC parameters and clinical course. Significance was assessed at least at the $p < 0.05$ level. In addition, the relationship between TT, NT, DD, lymphocyte, neutrophil, WBC, PLR, NLR parameters and the need for intensive care treatment (prognosis) of the patients was also examined by ROC analysis.

RESULTS

Within the scope of this study, the findings of a total of 99 individuals with 31 PCR negative healthy individuals (15-Female, and 16-Male), 68 positive PCR results (33 inpatients (18M, 15F) treated in the medical floor and 35 patients (19M, 16F) treated in the ICU) were evaluated. When the chronic diseases of the individuals were examined, it was found that 44.1% of COVID-19 PCR positive patients had no chronic disease, 22.1% had one and 33.8% had two or more chronic diseases. The most common of these diseases were 36.8% hypertension, 14.7% coronary artery disease, 11.8% diabetes, 5.8% chronic renal failure, 4.4% congestive heart failure and 4.4% COPD.

The results of CBC and Thiol analytes according to the groups are shown in table 1. In the control group, Total Thiol, NativeThiol, Dynamic Disulfide, hemoglobin, hematocrit, lymphocyte levels were higher than both inpatients and ICU patients on the contrary the NLR level was found to be low ($p < 0.05$). In inpatients group, lymphocyte count, Total Thiol, NativeThiol and Dynamic Disulfide levels are significantly higher than those treated in ICU unlike WBC (White Blood Cell), neutrophil and NLR rates were significantly lower ($P<0.05$). Neutrophil and PLR levels were found to be higher in patients treated in ICU than both the control group and the inpatients group ($P<0.05$), while there was no significant difference between the inpatients and the control groups ($P> 0.05$) (Table 1).

Table 1. CBC and thiol parameters according to groups

	Control	**Inpatient**	**ICU**
	Mean± SD	**Mean± SD**	**Mean± SD**
Age	52.6 ± 14.5	56.3 ± 15.8	70.4 ± 13.7
Total Thiol (umol/L)	531.9 ± 77	235.7 ± 107 [a b]	146.5 ± 83.5 [a]
Native Thiol (umol/L)	386.0 ± 66.8	180.4 ±76.5 [a b]	111.0 ± 62.5 [a]
Dynamic Disulfide (umol/L)	73.0 ± 9.05	27.6 ± 17.6 [a b]	17.8 ± 20.1 [a]
Dynamic Disulfide /Native Thiol (%)	19.3 ± 3.35	14.7 ± 6.7 [a]	19.7 ± 31.5 [a]
Dynamic Disulfide /Total Thiol (%)	13.8 ± 1.69	10.9 ± 3.97 [a]	11.4 ±6.9 [a]
Native/Total Thiol (%)	72.3 ± 3.38	78.0 ± 7.9 [a]	77.2 ± 13.8[a]
WBC (K/uL)	7.1 ± 1.26	6.0 ± 1.45 [a b]	8.9 ± 5.05
Hemoglobin (g/dL)	14.4 ± 1.56	12.6 ± 1.92 [a]	12.4 ± 1.74 [a]
Hematocrit (%)	43.6 ± 4.39	39.7 ± 6 [a]	38.5 ± 5.2[a]
Lymphocyte (K/uL)	2.3 ±0.66	1.56 ± 0.64 [a b]	0.9 ±0.46 [a]
Neutrophil (K/uL)	4.0 ±0.94	3.9 ± 1.5 [b]	6.7 ± 3.85 [a]
Platelet (K/uL)	231.3 ±51.2	200.5 ± 75.8 [a]	236.5 ± 128.1
NLO	1.8 ± 0.62	3.3 ± 3.33 [a b]	10.1 ± 7.25 [a]
PLO	105.3 ± 25.5	101.5 ± 149 [b]	334.4 ± 200.3 [a]

[a] according to control , [b] between the inpatient and ICU patients

When COVID-positive patients are grouped according to survival, Total Thiol, native thiol, dynamic disulfide, lymphocyte is significantly higher in the surviving patients on the contrary WBC, Neutrophil, PLR, NLR was significantly lower ($P<0.05$). When COVID positive patients are grouped according to the mechanical ventilation needed Total Thiol, native thiol, dynamic disulfide, lymphocyte is higher in patients who do not need ventilation unlike WBC, Neutrophil, PLR, NLR levels were found to be low ($P<0.05$). In individuals without chronic disease, the levels of Total Thiol, native thiol, dynamic disulfide, lymphocyte, hemoglobin, hematocrit were high, whereas WBC, Neutrophil, PLR, NLR levels were found to be low ($P<0.05$). TT, NT and DD levels are given in Figure 1 according to death and healing status, mechanical ventilation / spontaneous breathing and chronic disease status. Correlations between CBC and Thiol parameters are shown in Tables 2, 3 and 4.

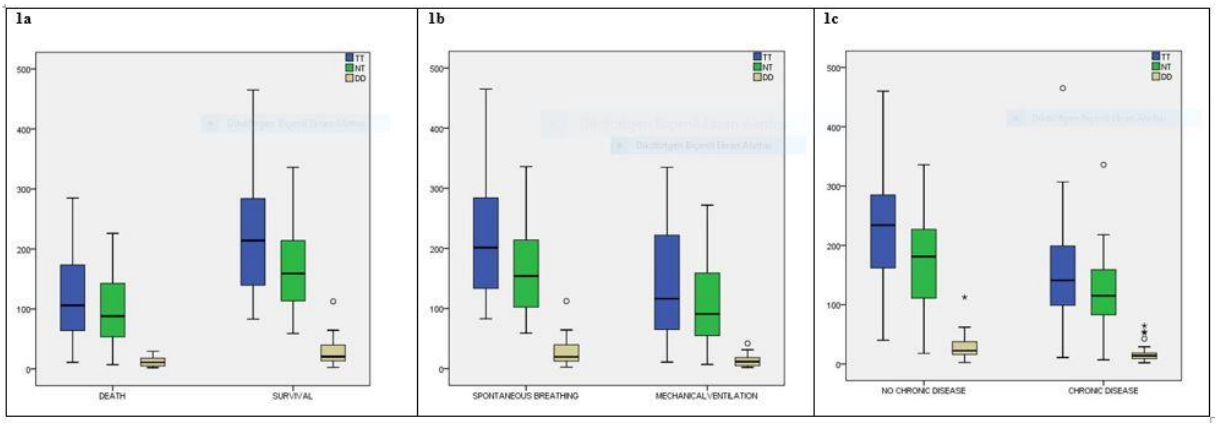

Figure 1. TT, NT, DD levels in PCR positive results 1a: survival status 1b: intubation status 1c: chronical disease status

Table 2. The relationship between thiol and cbc parameters in inpatient group

	Total Thiol	Native Thiol	Dynamic Disulfide	Dynamic Disulfide /NativeThiol (%)	Dynamic Disulfide /Total Thiol (%)	Native/Total Thiol (%)
WBC	-.131	-.088	-.074	-.052	-.052	.052
Hemoglobin	.522**	.576**	.337	-.064	-.064	.064
Hematocrit	.519**	.574**	.319	-.045	-.045	.045
Lymphocyte	.459**	.509**	.323	.024	.024	-.024
Neutrophil	-.326	-.307	-.236	-.096	-.096	.096
Platelet	.109	.073	.089	.062	.062	-.062
NLR	-.413*	-.440*	-.287	-.052	-.052	.052
PLR	-.566**	-.645**	-.347	.096	.096	-.096

Correlations are significant at the 0.05 and 0.01** levels*

Table 3. The relationship between thiol and cbc parameters in intensive care unit patients

	Total Thiol	Native Thiol	Dynamic Disulfide	Dynamic Disulfide /NativeThiol (%)	Dynamic Disulfide /Total Thiol (%)	Native/Total Thiol (%)
WBC	-.360*	-.347*	-.220	.243	.243	-.243
Hemoglobin	.193	.128	.165	.076	.076	-.076
Hematocrit	.141	.081	.095	.051	.051	-.051
Lymphocyte	.043	-.142	.265	.554**	.554**	-.554**
Neutrophil	-.272	-.217	-.176	.129	.129	-.129
Platelet	-.257	-.334*	-.131	.204	.204	-.204
NLR	-.120	.053	-.233	-.295	-.295	.295
PLR	-.117	-.008	-.227	-.277	-.277	.277

Correlations are significant at the 0.05 and 0.01** levels*

Table 4. The relationship between thiol and cbc parameters in the control group

	Total Thiol	Native Thiol	Dynamic Disulfide	Dynamic Disulfide /NativeThiol (%)	Dynamic Disulfide /Total Thiol (%)	Native/Total Thiol (%)
WBC	-.120	-.144	-.053	.100	.100	-.100
Hemoglobin	.483**	.481**	.141	-.424*	-.424*	.424*
Hematocrit	.514**	.510**	.152	-.440*	-.440*	.440*
Lymphocyte	-.155	-.180	-.133	.065	.065	-.065
Neutrophil	.008	-.002	.043	.039	.039	-.039
Platelet	-.379*	-.408*	-.103	.241	.241	-.241
NLR	.140	.151	.130	-.033	-.033	.033
PLR	-.226	-.230	-.003	.174	.174	-.174

Correlations are significant at the 0.05 and 0.01** levels*

In addition, we evaluated the relationship between the parameters, intensive care treatment requirement (prognosis) of the patients with ROC analysis. The parameters determining the need for intensive care of patients according to the increase and decrease in serum level are shown in Figure 2.

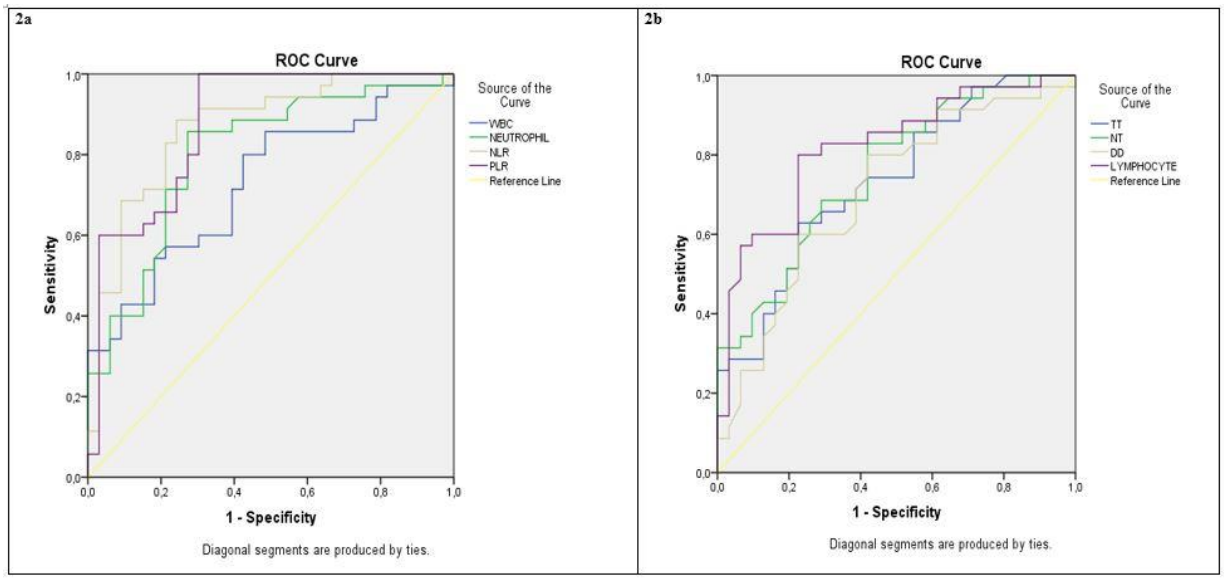

Figure 2. The signifcant relationships between the instensive care unit needed and prognosis 2a: WBC, nötrofil, NLR, PLR increased 2b: lenfosit, total tiol, native thiol, dinamic disulphite decreased

The cut-off values, area under the curve (AUC), likelihood ratio (LR), Confidence Interval (95%), sensitivity and specificity values of these parameters, which are thought to be used in terms of prognosis, are given in Table 5.

Table 5. Serum levels significant parameters for transfer to intensive care

	AUC (%95 CI)			p	Cut Off Value	Sensitivite	Spesifitite
	LB	Area	UB				
WBC *	.609	.728	.848	.001	6.92	.571	0.788
Neutrophil *	.695	.802	.908	.000	4.80	.714	0.788
NLR*	.779	.867	.954	.000	4.26	.829	0.788
PLR*	.793	.877	.961	.000	153.5	.800	0.727
Lymphocyte**	.718	.820	.922	.000	1.08	.800	0.774
Total Thiol **	.616	.735	.854	.001	167.5	.657	0.710
NativeThiol **	.642	.757	.871	.000	135.5	.686	0.710
Dynamic Disulfide**	.579	.706	.832	.004	14.25	.600	0.774

***significant increased, **significant decreased, AUC: Area under the curve, LR: likelihood ratio, %95 CI: %95 Confidence Interval, LB: Lower Bound UB: Upper Bound*

DISCUSSION

The balance of oxidant-antioxidant systems is important during the course of viral infections, both in the antimicrobial and the proinflammatory process. As a new indicator of oxidative stress, Thiol-disulfide balance has been studied in many different diseases. It provides valuable information about the processes that have important roles in maintaining the oxidant-antioxidant balance (11, 13, 15). The major thiols found in plasma are protein thiols and low molecular weight thiols including cysteine, cysteinylglycine, glutathione, homocysteine and γ-glutamylcysteine. Thiol groups are oxidized by disulfide bonds, which are reversibly oxidized by ROS. This mechanism mediates its antioxidant effects (16).

In this study we examined the relationship of thiol-disulfide balance with CBC parameters and its effect on the clinical course of COVID-19 patients. We found that TT, NT, DD, hemoglobin and hematocrit levels were lower in both inpatient and ICU COVID-19 patients compared to the control group. ICU patients showed lower TT, NT, DD and lymphocyte levels and higher WBC, neutrophils and NLR compared with inpatients. We also found that high TT, NT, DD and lymphocyte levels and low WBC, Neutrophil, PLR, NLR levels were significantly associated with reduced mortality and intubation requirement of the patients. Patients who did not have any underlying chronic diseases showed higher TT, NT, DD, lymphocyte, hemoglobin and hematocrit levels but lower WBC, Neutrophil, PLR and NLR levels compared to patients having underlying chronic diseases.

According to studies examining oxidant/antioxidant balance in infection and sepsis, oxidant parameters increased, and antioxidants

decreased, especially in ICU patients (17). It was also reported that the increased oxidant markers such as malondialdehyde in sepsis was related with the degree and mortality of sepsis (18). Esen et al.(19) reported that during infection, oxidant/antioxidant balance was shifted to the oxidant side, thus total thiol level, paroxonase and total antioxidant status decreased, total oxidant capacity and oxidative stress index increased. It also has been shown that antioxidant treatments had positive effects on the prognosis of infection and sepsis (20, 21). Consistent with these finding, our results showed that the thiol/disulfide balance was significantly disturbed in patients with COVID-19 infection.

Ayar et al. (22) found lower NT, TT, DD levels and higher ratio of DD/NT and DD/TT in pediatric-age group of sepsis patients compared to the control group. They stated that these parameters could be used as oxidative stress biomarkers. The researchers also reported that there was no significant difference between the thiol-disulfide balance and survival of the patients. The changes in TT, NT and DD levels in our patients with COVID-19 infection were consistent with their findings in pediatric sepsis patients. However, the higher TT, NT, DD levels were related with survival of patients and clinical course of COVID-19 infection in our study. Aydogan et al. (23) have reported that lower NT, TT, NT / TT ratio and higher DD / TT ratio could be used in early diagnosis of neonatal sepsis. Although TT and NT levels obtained in our study were consistent with their findings, DD levels were low in our patients. This contradiction may result from the differences in patient's age groups. It also might be related with ethio-pathogenesis of diseases.

Kara et al. (24) have compared the thiol / disulfide balance in bacterial and viral infections in their study. Their results showed that NT, TT, NT / TT ratios were lower in both infections compared to the control group, and DD / NT ratios were higher. They also stated that DD levels were lower in bacterial infections than viral infections. Additionally, they found that the WBC count were negatively correlated with NT, TT levels. In our study, we found that TT and NT levels were positively correlated with lymphocyte levels and negatively correlated with NLR and PLR in COVID-19 patients treated at the medical floor. We also found that NT and TT levels were negatively correlated with WBC in ICU patients.

Liu et al. (25) have demonstrated that viral proteins attack the beta chain of hemoglobin, allowing the heme part to decompose into iron and porphyrin in COVID-19 infection. Therefore both the amount and the oxygen carrying capacity of hemoglobin are reduced in COVID-19 patients. Free iron released in this process can also cause oxidative damage by Fenton reactions. Both increased free iron and increased oxidative status also affect T lymphocytes (26, 27). In the experimental studies protein and lipid oxidation were demostrated in erythrocytes due to ROS and membrane damage was observed in erythrocytes by electron microscopy. Similarly, cytotoxic and genotoxic effects have been observed in lymphocytes as a result of oxidative DNA damage (28, 29). It is known that erythrocyte membrane damage due to ROS increases in disease states and this results in a decrease in hemoglobin levels by increasing intravascular hemolysis (30). In our study, we found that TT and NT levels were significantly correlated with lymphocyte, hemoglobin, hematocrit levels in inpatient group, and hemoglobin and hematocrit levels in the control group. These findings suggest that the decrease in hemoglobin and lymphocyte levels in our patients may be due to the increased oxidative stress in COVID-19 infection.

In our study, we demonstrated low levels of TT, NT, DD, lymphocytes, and high levels of WBC, Neutrophils, PLR, and NLR in both patients who died or intubated due to Covid 19 infection. By evaluating ROC analysis, we found that TT, NT, DD levels and CBC parameters showed high sensitivity and specificity for determining requirement of patients to intensive care treatment. It has been reported that the NLR is an independent risk factor of in-hospital mortality for COVID-19 patients. Each unit increase in NLR increases the mortality risk by 8% (31, 32). There are several studies showing conflicting results about association of CBC parameters and NLR with Covid 19 infection (33-35) in the literature. Some of them are consistent with our results and some of them are not. For the first time, we reported optimal cut off values of TT, NT, DD levels and CBC parameters such as WBC, neutrophil counts, NLR, PLR for predicting requirement of patients to intensive care treatment. We also found that high TT, NT, DD and lymphocyte levels and low WBC, Neutrophil, PLR, NLR levels were significantly associated with reduced mortality and intubation requirement of the patients. Therefore, we think that the results of our study will contribute significantly to the literature on these subjects and will provide preliminary data for further research.

As conclusion, the results of this study clearly showed that the thiol-disulfide balance is disturbed in COVID-19 disease for the first time in the literature. Monitoring the thiol-disulfide balance may be beneficial in the follow-up of the patients. The main limitation of this study is its relatively small sample size and further studies with larger sample sizes are needed.

Ethics approval: This study was performed in line with the principles of the Declaration of Helsinki. Ethics committee approval was obtained from XX University Faculty of Medicine Clinical Research Ethics Committee for the study with the decision dated 27/05/2020 and numbered 110.

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