Original Article

Relationship between Concentrations of Oxygen and Levels of Oxidative Stress in Patients Receiving Oxygen Therapy for Severe COVID-19 Pneumonia

Salih Özenç[1], Pınar Yıldız Gülhan[1]* , Merve Alpay[2], Şule Yıldız[1], Nevra Ezgi Yaşlı[1] and Peri Meram Arbak[1]

[1]*Department of Chest Diseases, Faculty of Medicine, Duzce University, Duzce, Türkiye;* [2]*Department of Biochemistry, Faculty of Medicine, Duzce University, Duzce, Türkiye*

Abstract

Background and objectives: Severe COVID-19 pneumonia often requires high concentrations of oxygen, which can potentially lead to oxidative stress and lung injury. This study aimed to investigate the impact of different oxygen therapy modalities on oxidative stress by comparing malondialdehyde (MDA) levels, an oxidative stress marker, and glutathione (GSH), an antioxidant marker, in patients with severe COVID-19 pneumonia.

Methods: This study included 50 patients with COVID-19 pneumonia who received oxygen therapy using a reservoir mask at ≥15 L/m, high-flow oxygen therapy at 60 L/m, or oxygen therapy with noninvasive mechanical ventilation at fraction of inspired O_2 (FiO_2) levels of ≥60%. GSH and MDA levels were measured 48 h after starting oxygen therapy at $FiO_2 \geq 60\%$ and 48 h after switching to nasal cannula oxygen therapy at 2–4 L/m.

Results: Overall, 60% (n = 30) of the patients were men, and 40% (n = 20) were women. In patients with accompanying hypertension, MDA levels, which were higher during oxygen therapy at $FiO_2 \geq 60\%$, decreased significantly after switching to nasal cannula oxygen therapy at 2–4 L/m ($p = 0.046$). A significant difference in MDA was not found after switching to lower oxygen flow ($p = 0.064$) in patients with underlying diabetes mellitus. GSH levels in patients with underlying diabetes mellitus were higher during oxygen therapy at $FiO_2 \geq 60\%$ and decreased significantly after switching to nasal cannula oxygen therapy at 2–4 L/m ($p = 0.021$).

Conclusions: This study compared MDA and GSH levels among patients receiving oxygen therapy at high and low concentrations for severe COVID-19 pneumonia. The results revealed that patients who died of COVID-19 pneumonia had significantly higher mean MDA levels than those who survived. In patients with underlying HT, MDA levels, which were higher during oxygen therapy at $FiO_2 \geq 60\%$, decreased during nasal oxygen therapy at 2–4 L/m; this difference was significant. The increase in serum MDA levels during high-flow oxygen therapy and the decrease during low-flow therapy in patients with COVID-19 pneumonia accompanied by hypertension suggest that oxidative stress due to hyperoxia should be taken into consideration.

Introduction

Coronavirus disease 2019 (COVID-19) was first identified in Wu-

han, China, in December 2019. Since then, it has become a severe health problem, resulting in a global crisis with economic, social, and psychological impacts.[1] Patients with COVID-19 pneumonia range from outpatients with mild disease to those with severe respiratory failure who require intensive care.[2]

Oxygen therapy at high concentrations is required to treat severe COVID-19 pneumonia. It is crucial to understand that oxygen exerts specific biochemical and physiological effects at effective doses within an adequate range but has well-known side effects at extremely high doses. Oxygen supplementation administered at high concentrations to patients with COVID-19 pneumonia leads to hyperoxia. When excessive oxygen is administered, the lungs

are the first organ affected. Exposure to 100% oxygen has been reported to increase levels of proinflammatory cytokines and the number of inflammatory cells, such as macrophages and neutrophils. This further causes oxidative stress and inflammation in animal lungs, which is evidence of tissue damage.[3–5]

The study by Nagato *et al*.[3] examined lung injury caused by hyperoxia and found that hyperoxia first induces oxidative stress in the lungs, followed by inflammation and tissue damage. Malondialdehyde (MDA), an indicator of oxidative stress, was found to increase in the lungs of mice at 12 h and 24 h after exposure to hyperoxia, indicating that hyperoxia induces oxidative stress early on. At 48 h of hyperoxia, MDA levels returned to normal, but tissue damage was evident in the lungs during this period, suggesting that early oxidative stress may lead to permanent damage. Glutathione (GSH), an antioxidant that protects cells from oxidative damage, decreased in the lungs of mice exposed to hyperoxia at 12 h and 24 h, suggesting that hyperoxia may exacerbate lung damage by depleting GSH. In conclusion, this study shows that MDA and GSH play important roles in hyperoxia-induced lung injury, and the levels of these substances may be markers of early lung injury.

Oxidative stress develops when the rate of free radical generation exceeds the antioxidant defense capacity, resulting in the toxic effects of free radicals.[6,7] Free radical species are important physiological components in biological homeostasis.[8,9]

Several products, such as MDA, 4-hydroxy-2-nonenal, and 15(S)-8-iso-PGF2a, are well-known consequences of lipid peroxidation. In particular, MDA is a widely used marker of oxidative stress.[10] Studies have shown that MDA levels are increased in various diseases, such as cancer,[11] diabetes,[12] chronic obstructive pulmonary disease (COPD),[13] hypertension (HT),[14] ARDS,[15] atherosclerosis, and neurodegenerative diseases such as Alzheimer's and Parkinson's, compared to healthy individuals.[16]

GSH, a water-soluble tripeptide composed of glutamate, cysteine, and glycine, contains a thiol group that acts as a potent reducing agent. This makes GSH the most abundant low-molecular-weight thiol within cells, reaching millimolar concentrations in some tissues. As a crucial antioxidant, GSH participates in the detoxification of various electrophilic compounds and peroxides, catalyzed by glutathione S-transferases and glutathione peroxidases.[17,18]

Numerous studies have demonstrated a decrease in GSH levels in individuals infected with COVID-19.[19–21] Khanfar and Al Qaroot highlighted that various risk factors associated with COVID-19's high mortality rate are linked to low GSH levels or impaired GSH metabolism.[22] Conditions such as aging, hypertension, ischemic heart disease, diabetes, and chronic lung disease are associated with decreased baseline GSH levels, while smoking, obesity, alcoholism, renal failure, cancers, and other chronic diseases are linked to disruptions in GSH homeostasis, impairing glutathione functionality. Although these conditions may not increase the risk of contracting COVID-19, they contribute to a more severe disease course and increased mortality rates upon infection.[22,23]

Investigating the effects of oxygen therapy on oxidant and antioxidant levels, as well as examining the impact of oxidative stress on recovery from COVID-19 pneumonia, may facilitate the development of tailored oxygen therapy strategies optimized for individual patients.

This study aimed to examine the oxidant and antioxidant status in patients with hypoxemia receiving oxygen therapy using non-rebreather reservoir masks or high-flow oxygen devices. Furthermore, it aimed to determine the oxidant and antioxidant status in patients receiving high-concentration oxygen therapy for the treatment of COVID-19 pneumonia and to assess the benefits and harms of oxygen therapy by comparing the clinical status of these patients.

Materials and methods

This study was conducted in accordance with the ethical principles of the Declaration of Helsinki. Written informed consent was obtained from each patient prior to enrollment. The study protocol was approved by the Clinical Research Ethics Committee of Düzce University (decision no. 2020/275, dated January 4, 2021).

Study design and population

This prospective cohort study included 50 patients who were hospitalized for severe COVID-19 with respiratory failure in the pandemic ward of Düzce University Medical Faculty Hospital between January 2021 and February 2022. Of these 50 patients, 30 were discharged after treatment, while the remaining 20 died of COVID-19 (Fig. 1). Written consent was obtained from all patients after explaining the nature, content, and scope of the study.

Patient parameters, including age, sex, and smoking status, were collected, and body mass index, waist circumference, and hip circumference were measured. Past medical records and drug exception reports were examined for each patient to extract and record co-occurring conditions related to COVID-19, as well as any regularly prescribed medications. Computerized tomography was performed before hospitalization to record the radiographic assessment of COVID-19 severity, patterns of involvement, and the number of lobes involved.

For each patient, the following parameters were recorded: peripheral capillary oxygen saturation (SpO_2) levels and respiratory rate measured after 48 h of oxygen therapy at $\geq$15 L/m for those using a non-rebreather mask, or at a fraction of inspired O_2 (FiO_2) $\geq$60% for those using noninvasive mechanical ventilation or a high-flow nasal cannula. Laboratory tests were performed to measure levels of lactate dehydrogenase (LDH), ferritin, D-dimer, and C-reactive protein (CRP). MDA and GSH levels were determined using blood samples collected at that time. Antibiotics, antivirals, anti-inflammatory medications, and immunomodulatory therapies administered during hospitalization were also recorded.

For the 30 patients who completed treatment, the following parameters were recorded: SpO_2 levels and respiratory rates after 48 h of oxygen therapy at 2–4 L/m via nasal cannula prior to discharge. Laboratory tests were again performed to determine LDH, ferritin, D-dimer, and CRP levels, and MDA and GSH levels were measured using blood samples collected at that time.

Measurement of malondialdehyde and glutathione levels

Samples collected from patients were stored in ethylenediamine tetraacetic acid tubes, centrifuged at 3,000 rpm for 15 m, and the supernatant was collected in Eppendorf tubes. Each sample was stored at −80°C until analysis. Measurements were performed at room temperature. A double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) was performed to determine MDA and GSH levels in the samples.

Serum MDA levels were measured using the Sunred Human Malondialdehyde ELISA Kit (catalog no.: 201-12-5380), which uses the double antibody ELISA method to quantify MDA levels. According to the prescribed procedure, MDA samples were added to wells pre-coated with MDA monoclonal antibodies. Biotin and streptavidin-horseradish peroxidase (streptavidin-HRP) solutions were then added to form immune complexes. The wells
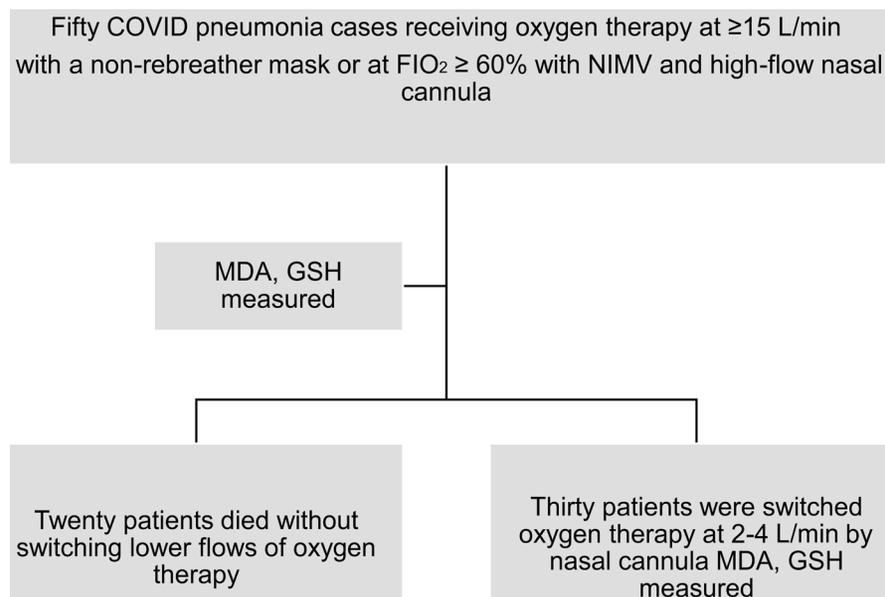
Fig. 1. Characteristics of the study population. FiO_2, the fraction of inspired O_2; GSH, glutathione; MDA, malondialdehyde; NIMV, noninvasive mechanical ventilation.

were covered with a sealing film and incubated at 37°C for 60 m. After incubation, the sealing film was removed, and the wells were washed to eliminate unbound enzymes. Chromogen solutions A and B were added, causing the solution to turn blue. The samples were incubated for 10 m at 37°C. After incubation, a stop solution was added, changing the color from blue to yellow. There was a positive correlation between the shade of color and human MDA levels. The yellow shades indicated the optical density of the wells, which was measured at a wavelength of 450 nm. After applying standard dilutions to empty wells and treating them with chromogen A, chromogen B, and stop solution, standard values were obtained. Using these standard and optical density values, a standard curve was constructed. The curve was then used to calculate the regression equation, which facilitated the calculation of MDA levels corresponding to the optical density of each MDA sample. MDA levels were calculated in nmol/mL. After completing the analysis, the data achieved as nm were converted into concentrations using the curve expert program, based on the standard results of the kit studied. A geometric fit was applied at 450 nm absorbance for the MDA test (R = 0.97649) and the GSH test (R = 0.98704) to ensure validity (Fig. 2).

Serum GSH levels were measured using the Sunred Human Glutathione ELISA Kit (catalog no.: 201-12-1463). The kit employs a double antibody ELISA method to determine GSH levels in the samples. The test was performed according to the prescribed procedure. GSH samples were added to wells pre-coated with GSH monoclonal antibodies. Biotin and streptavidin-HRP solutions were then added to form immune complexes. The wells were covered with a sealing film and incubated at 37°C for 60 m. After incubation, the sealing film was removed, and the wells were washed to eliminate unbound enzymes. Chromogen solutions A and B were added, causing the solution to turn blue. The samples were incubated for an additional 10 m at 37°C. After this incubation, a stop solution was added to each well, changing the color from blue to yellow. There was a positive correlation between the shade of color and human GSH levels. The yellow shades indicated the optical density of the wells, which was measured at a

wavelength of 450 nm. After applying standard dilutions to empty wells and treating them with chromogen A, chromogen B, and the stop solution, standard values were obtained. Using these standard values and optical density measurements, a standard curve was constructed. This curve was used to calculate the regression equation, which in turn was used to calculate the GSH levels corresponding to the optical density of each sample. GSH levels were calculated in µmol/L.

Inclusion criteria

Written informed consent was obtained from all 50 patients after providing information about the nature, content, and scope of the study. This study included patients who tested positive for COVID-19 using reverse transcription polymerase chain reaction and were undergoing treatment in the pandemic ward, receiving oxygen therapy with a non-rebreather reservoir mask at ≥15 L/m, noninvasive mechanical ventilation, or a high-flow nasal cannula at $FiO_2 \geq 60\%$.

Exclusion criteria

Patients who refused to participate in the study or provide informed consent, those under 18 years of age, and pregnant women were excluded.

Statistical analysis

Statistical analysis was performed using SPSS 21. Descriptive statistics (mean, standard deviation) were calculated based on the data type. Independent samples t-tests were used for intergroup comparisons of variables that met parametric test assumptions. The Mann–Whitney U test was used for variables that did not meet these assumptions. Categorical variables were compared using Fisher's exact test and Pearson's chi-square test. The Wilcoxon test was used to calculate the difference between pre-test and post-test scores of quantitative variables. Univariate analysis was used to detect the effect of survival on MDA and LDH levels. Statistical significance was set at $p < 0.05$.
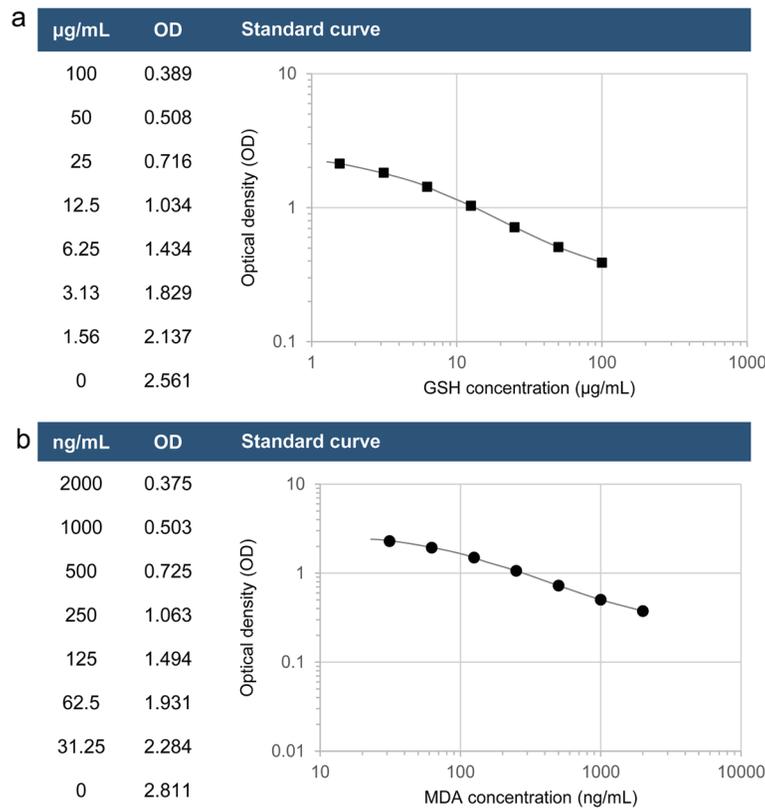
Fig. 2. The calibration and validation procedures for the ELISA method. ELISA, enzyme-linked immunosorbent assay; GSH, glutathione; OD, optical density.

Results

The mean age of the patients was 66.0 ± 15.2 years (range: 40–93 years). Sixty percent of the patients were men, and 52% were smokers. The most common underlying diseases were HT, diabetes mellitus (DM), ischemic heart disease, COPD, congestive heart failure, benign prostatic hyperplasia, hypothyroidism, and chronic renal failure (Table 1).

Patients receiving oxygen therapy at $FiO_2 \geq 60\%$ had SpO_2 levels of approximately 93% and a respiratory rate of 23/m. Their mean LDH, ferritin, D-dimer, and CRP levels were above normal reference values (Table 2).

In total, 20 patients died. Table 3 shows the differences in demographic data and oxidative and inflammatory markers between survivors and non-survivors (Table 3).

The mean MDA level (10.12 *vs.* 6.65, $p = 0.001$) and LDH level (515.55 *vs.* 374.86, $p = 0.005$) of deceased patients were significantly higher than those of survivors. Although the mean ferritin, D-dimer, and CRP levels of deceased patients were higher than those of survivors, the differences were not statistically significant.

Univariate analysis showed that survival had an independent and significant effect on MDA levels during high-flow oxygen administration (Adjusted R^2: 0.137, F: 8.796, $p = 0.005$). Similarly, univariate analysis showed that survival had an independent and significant effect on LDH levels during high-flow oxygen administration (Adjusted R^2: 0.125, F: 7.995, $p = 0.007$). Patients with higher MDA and LDH levels during high-flow oxygen administration had higher mortality rates.

The rate of comorbid hypertension in deceased patients was significantly higher than in survivors (75% *vs.* 43.3%, $p = 0.042$).

Table 1. Characteristics of the patients

	n	%
Gender		
Female	20	40.0
Male	30	60.0
Smoker		
Yes	26	52.0
No	24	48.0
Disease		
COPD	13	26.0
Asthma	4	8.0
Hypertension	28	56.0
Diabetes mellitus	16	32.0
Congestive heart failure	6	12.0
Ischemic heart disease	13	26.0
Chronic kidney disease	4	8.0
Benign prostate hyperplasia	5	10.0
Hypothyroidism	5	10.0
More than one disease		
Yes	32	64.0
No	18	36.0

COPD, chronic obstructive pulmonary disease.

Table 2. Oxidative stress and biochemical parameters during the oxygen therapy treatment period with ≥60% FiO₂

	Mean (SD)	Median (IQR)
MDA (nmol/mL)	8.04 (0.61)	6.95 (3.18)
GSH (Umol/L)	3.90 (0.58)	2.92 (2.05)
SpO₂ (%)	93 (2.67)	93 (3.25)
Respiratory rate/m	23 (3.08)	23 (5.00)
LDH (U/L)	431 (184)	398 (219)
Ferritin (ng/mL)	691 (544)	517 (816)
D-dimer (µg/mL)	2.14 (2.56)	1.10 (1.91)
CRP (mg/dL)	7.81 (6.29)	6.99 (8.06)

CRP, C-reactive protein; GSH, glutathione; IQR, interquartile range; LDH, lactate dehydrogenase; MDA, malondialdehyde; SD, standard deviation; SpO₂, pulse oximeter oxygen saturation.

Additionally, the rate of comorbidities involving more than two diseases was significantly higher in deceased patients compared to survivors (85% *vs.* 50%, $p = 0.016$). No significant differences were found between deceased and surviving patients regarding gender, smoking status, presence of other diseases, or severity of radiological involvement.

Table 4 shows the results of the repeated tests in the 30 survivors for respiratory rate as well as MDA, GSH, LDH, ferritin, D-dimer, CRP, and SpO₂ levels while receiving nasal cannula oxygen therapy at 2–4 L/m.

During nasal cannula oxygen therapy at 2–4 L/m, the mean SpO₂ levels increased significantly, while the mean respiratory rate and LDH, ferritin, and CRP levels decreased significantly. MDA and GSH levels, which were higher during oxygen therapy at FiO₂ ≥ 60%, decreased during nasal cannula oxygen therapy at 2–4 L/m, but the difference was not statistically significant.

In patients with underlying HT, MDA levels, which were higher during oxygen therapy at FiO₂ ≥ 60%, decreased significantly during nasal oxygen therapy at 2–4 L/m (from 6.26 to 5.57 nmol/mL, $p = 0.046$). In patients with underlying DM, GSH levels, which were higher during oxygen therapy at FiO₂ ≥ 60%, decreased significantly during nasal oxygen therapy at 2–4 L/m (from 3.95 to 3.05, $p = 0.021$). In patients with radiologically detected severe involvement, GSH levels, which were higher during oxygen therapy at FiO₂ ≥ 60%, decreased significantly during nasal cannula oxygen therapy at 2–4 L/m (from 3.61 to 2.94, $p = 0.039$). Analysis of the results according to sex, age, smoking status, presence of COPD, and mild to moderate radiological involvement revealed no significant differences in MDA and GSH levels between patients receiving oxygen therapy at different concentrations. How-

Table 3. Comparison of demographic data and oxidative, inflammatory markers of survivors and dead patients

	Survivors (n = 30)		Non-Survivors (n = 20)		*p*
	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)	
Age (years)	65.70 (3.12)	66.00 (30.25)	66.55 (12.18)	66.00 (14.00)	0.851
BMI	30.50 (1.22)	28.95 (7.08)	28.93 (5.41)	29.05 (5.80)	0.482
MDA(nmol/mL)	6.65 (3.26)	5.73 (3.10)	10.12 (5.01)	8.03 (5.42)	0.001
GSH(Umol/L)	4.56 (5.09)	3.08 (2.00)	2.91 (1.70)	2.55 (1.63)	0.076
LDH(U/L)	374.86 (29.09)	343.00(184.25)	515.55 (190.49)	485.50 (314.00)	0.005
Ferritin(ng/mL)	579.44 (88.35)	461.00(626.93)	858.49 (598.93)	724.75 (950.75)	0.075
D-dimer(µg/mL)	1.78 (0.40)	0.92 (1.83)	2.67 (3.00)	1.59 (3.13)	0.198
CRP(mg/dL)	6.20 (0.81)	6.31 (6.78)	10.23 (7.85)	7.64 (10.15)	0.078

BMI, body mass index; CRP, C-reactive protein; GSH, glutathione; LDH, lactate dehydrogenase; MDA, malondialdehyde.

Table 4. Comparison of treatment results of 30 survivors with ≥60% FiO₂ oxygen therapy and nasal 2–4 Lt/m oxygen therapy

	≥60% FiO₂ oxygen therapy		Nasal 2–4 Lt/m oxygen therapy		*p*
	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)	
MDA (nmol/mL)	6.65 (0.59)	5.73 (3.10)	6.38 (0.50)	5.76 (2.42)	0.734
GSH (Umol/L)	4.56 (0.93)	3.08 (2.00)	3.96 (0.94)	2.59 (1.43)	0.113
SpO₂ (%)	92.63 (2.67)	92.00 (3.25)	94.36 (1.90)	94.50 (3.00)	0.001
Respiratory rate/m	22.96 (2.85)	23.50 (6.00)	16.90 (2.53)	17.50 (4.00)	<0.001
LDH (U/L)	374.86 (159.35)	343.00 (184.25)	281.83 (72.18)	278.00 (83.00)	0.006
Ferritin (ng/mL)	579.44 (483.91)	461.00 (626.93)	372.49 (252.55)	292.50 (407.05)	0.006
D-dimer (µg/mL)	1.78 (2.20)	0.92 (1.83)	1.25 (1.27)	0.85 (1.06)	0.077
CRP (mg/dL)	6.20 (4.46)	6.31 (6.78)	1.72 (2.37)	0.62 (2.14)	<0.001

CRP, C-reactive protein; GSH, glutathione; IQR, interquartile range; LDH, lactate dehydrogenase; MDA, malondialdehyde; SD, standard deviation; SpO₂, pulse oximeter oxygen saturation.

ever, patients aged <60 years showed a notable, though not statistically significant, decrease in MDA levels (from 7.42 to 6.23, $p = 0.117$). Non-smokers showed a noticeable decrease in GSH levels (from 4.93 to 3.09, $p = 0.093$). Patients without COPD exhibited a marked decrease in GSH levels (from 4.98 to 4.30, $p = 0.153$), and those without HT showed a decrease in GSH levels (from 5.53 to 4.58, $p = 0.136$) (Table 5).

Discussion

This study compared MDA and GSH levels among patients receiving oxygen therapy at high and low concentrations for severe COVID-19 pneumonia. The results revealed that patients who died of COVID-19 pneumonia had significantly higher mean MDA and LDH levels than those who survived. In patients with underlying HT, MDA levels, which were higher during oxygen therapy at $FiO_2 \geq 60\%$, decreased during nasal oxygen therapy at 2–4 L/m; this difference was significant ($p = 0.046$). In patients with underlying DM, GSH levels, which were higher during oxygen therapy at $FiO_2 \geq 60\%$, decreased significantly during nasal oxygen therapy at 2–4 L/m ($p = 0.021$).

Alveolar hyperoxia caused by high FiO_2 demand can exacerbate lung injury by leading to several adverse effects, such as oxidative stress, inflammation, apoptosis, and surfactant dysfunction in the alveoli. Particularly in COVID-19 patients, hyperoxia treatment in conditions such as acute lung injury and respiratory distress syndrome can increase lung inflammation, trigger reactive oxygen species production, and cause cellular damage. This can lead to prolonged hospitalization, secondary infections, and even death. Furthermore, hyperoxia may facilitate SARS-CoV-2 entry into cells by increasing the expression of the angiotensin-converting enzyme-2 protein. Caution is essential during treatment, especially in elderly COVID-19 patients with pre-existing health conditions, as prolonged hyperoxia use may lead to adverse outcomes.[24,25]

Numerous studies have investigated lung injury caused by hyperoxia exposure. Davis *et al*.[26] found increased alveolo-capillary permeability in volunteers breathing 95% oxygen for 17 h. Aggarwal *et al*.[27] in a retrospective analysis of 10 randomized ARDS trials, found that oxygen levels above targeted levels ($PaO_2 > 80$ mm Hg) were associated with increased mortality. Fang *et al*.[28] found that angiotensin-converting enzyme-2 was protective in an animal model of hyperoxic lung injury through inhibition of the proinflammatory NF-κB pathway and activation of the antioxidant Nrf2/HO-1/NQO1 pathway. Baleeiro *et al*.[29] demonstrated the negative effects of hyperoxia (FiO_2 95%) on alveolar macrophage bactericidal function in a mouse model of Klebsiella pneumoniae infection. These studies highlight different mechanisms through which hyperoxia contributes to lung injury.

In a study evaluating the effect of hyperoxia on oxidative stress and assessing MDA as a potential marker for this stress, patients undergoing abdominal surgery were randomly assigned to two groups receiving either 40% or 80% oxygen. Blood samples were collected at various times during and after surgery to measure oxidative stress markers. The findings indicated that in the 80% oxygen group, MDA levels at 24 h post-surgery were significantly higher compared to both baseline values and the 40% oxygen group. Additionally, antioxidant defenses were reduced in this group. Hyperoxia weakens antioxidant defenses, leading to increased lipid peroxidation and consequently elevated MDA levels. This indicates oxidative stress that can potentially cause cellular damage.[30] In our study, all deceased patients had received high-

flow oxygen therapy, and MDA levels were significantly higher in deceased patients compared to survivors.

Hypertension is a major risk factor for endothelial dysfunction and atherosclerosis. These subclinical conditions may, therefore, affect cardiovascular outcomes in patients with COVID-19.[31] The relationship between HT and COVID-19 may involve common inflammatory pathways. Indeed, numerous studies support the hypothesis that HT is associated with immune activation and oxidative stress, including ROS (reactive oxygen species) production, increased nicotinamide adenine dinucleotide phosphate oxidase activity, cell migration, and adhesion to the endothelial surface.[32] In our study, patients with HT had higher MDA levels while receiving oxygen therapy at $FiO_2 \geq 60\%$ than while receiving nasal cannula oxygen therapy at 2–4 L/m ($p = 0.046$).

Some studies have examined the relationship between HT and the levels of MDA and GSH.[33–35] In their study, which recruited 25 normotensive and 40 hypertensive patients, Ahmad *et al*.[35] investigated oxidant and antioxidant levels in both groups. They further categorized hypertensive patients into three subgroups: pre-HT, HT stage I, and HT stage II. Blood pressure was measured, and serum levels of oxidative stress markers were estimated. Patients with HT stage I and stage II were administered antihypertensive therapy for six months, during which their blood pressure was strictly regulated, eventually becoming normotensive. After six months, the levels of oxidative stress markers were re-estimated. MDA levels were significantly higher in the HT stage I and stage II groups than in the control group. In contrast, the levels of antioxidant enzymes (SOD, catalase, and peroxidase) were significantly lower in the pre-HT, HT stage I, and HT stage II groups compared to the control group. Antioxidant enzyme levels increased significantly after blood pressure returned to normal at the end of six months of strict regulation and antihypertensive therapy.

Data from the National Health Commission of China showed that 35% of patients with COVID-19 also had HT. A study comparing 126 patients with COVID-19 and pre-existing HT to 125 age- and sex-matched patients with COVID-19 without HT found that hypertensive patients had a 21.3% higher rate of severe SARS-CoV-2 infection and a higher mortality rate (6.4% *vs*. 10.3%).[36] A study with a cohort of 191 COVID-19 patients from Wuhan, China, found that 48% of survivors had HT.[37] Another study reported that, in a group of 138 hospitalized patients, 58% of those admitted to the intensive care unit had HT, which was identified as a significant risk factor associated with worse clinical outcomes.[38] In line with these results, our study also found that patients who died had significantly higher rates of HT (75.0% *vs*. 43.3%) and ischemic heart disease (45.0% *vs*. 13.3%). Since the early days of the pandemic, HT has been recognized as a risk factor for severe COVID-19 infection. One possible reason for this association could be the increased oxidative stress due to high-flow oxygen therapy. In our study, we observed a significant decrease in MDA levels (an oxidative stress marker) when hypertensive patients receiving high-flow oxygen therapy ($FiO_2 \geq 60\%$) were transitioned to low-flow oxygen (2–4 L/m nasal cannula). Furthermore, deceased patients exhibited higher MDA levels compared to survivors, indicating the potential impact of MDA on mortality. The mechanism underlying the increase in MDA levels with high-flow oxygen therapy in hypertensive patients should be further investigated in future studies.

A study by Gawlik *et al*. examining antioxidant levels in patients with DM found that GSH levels were lower in the DM group than in the control group. They also indicated that patients with type 2 diabetes had lower intracellular GSH concentrations,

Table 5. MDA and GSH values measured during treatment with ≥60% FiO$_2$ oxygen therapy and nasal 2–4 Lt/m oxygen therapy

	≥60% FiO$_2$ oxygen therapy		Nasal 2–4 Lt/Dk oxygen therapy		*p*
	Mean (SD)	**Median (IQR)**	**Mean (SD)**	**Median (IQR)**	
Male					
MDA (nmol/mL)	6.64 (1.03)	5.59 (3.19)	5.99 (0.68)	5.50 (1.42)	0.363
GSH (Umol/L)	5.08 (1.76)	2.72 (1.88)	4.37 (1.83)	2.61 (1.54)	0.394
Female					
MDA (nmol/mL)	6.66 (0.64)	5.88 (2.04)	6.76 (0.75)	6.63 (2.90)	0.609
GSH (Umol/L)	4.03 (0.66)	3.20 (2.39)	3.56 (0.56)	2.58 (1.99)	0.173
Age					
<60 years					
MDA (nmol/mL)	7.42 (1.24)	6.86 (4.72)	6.23 (0.89)	5.75 (2.60)	0.117
GSH (Umol/L)	4.20 (1.02)	3.22 (1.94)	5.32 (2.30)	2.59 (2.59)	0.239
≥60 years					
MDA (nmol/mL)	6.14 (0.55)	5.57 (2.79)	6.48 (0.61)	5.76 (2.12)	0.306
GSH (Umol/L)	4.80 (1.41)	3.08 (2.39)	3.06 (0.33)	2.75 (1.24)	0.157
Smoker					
Yes					
MDA (nmol/mL)	6.75 (1.21)	5.49 (4.96)	5.98 (0.81)	5.38 (2.09)	0.422
GSH (Umol/L)	4.07 (0.95)	3.01 (2.23)	5.10 (2.13)	2.61 (2.49)	0.650
No					
MDA (nmol/mL)	6.58 (0.53)	5.99 (2.04)	6.68 (0.65)	6.12 (2.42)	0.758
GSH (Umol/L)	4.93 (1.49)	3.14 (2.04)	3.09 (0.34)	2.55 (1.37)	0.093
COPD (+)					
MDA (nmol/mL)	5.53 (0.84)	5.02 (4.11)	5.17 (0.37)	5.44 (1.50)	0.753
GSH (Umol/L)	2.85 (0.53)	2.47 (1.99)	2.62 (0.51)	2.66 (2.35)	0.345
COPD (−)					
MDA (nmol/mL)	6.93 (0.71)	5.93 (2.34)	6.68 (0.61)	6.12 (2.73)	0.886
GSH (Umol/L)	4.98 (1.14)	3.17 (2.22)	4.30 (1.16)	2.59 (1.45)	0.153
Hypertension (+) n = 13					
MDA (nmol/mL)	6.26 (0.41)	6.64 (2.63)	5.57 (0.31)	5.38 (1.74)	0.046
GSH (Umol/L)	3.28 (0.31)	3.36 (2.04)	3.15 (0.40)	2.96 (1.26)	0.685
Hypertension (−) n = 17					
MDA (nmol/mL)	6.95 (1.01)	5.56 (4.68)	7.00 (0.84)	6.43 (2.93)	0.939
GSH (Umol/L)	5.53 (1.60)	3.03 (4.64)	4.58 (1.64)	2.55 (2.07)	0.607
Diabetes mellitus(+)					
MDA (nmol/mL)	6.85 (0.80)	5.88 (2.30)	6.66 (0.97)	5.45 (1.86)	0.657
GSH (Umol/L)	3.95 (0.71)	2.86 (1.96)	3.05 (0.30)	2.55 (1.24)	0.021
Diabetes mellitus (−)					
MDA (nmol/mL)	6.54 (0.83)	5.52 (3.19)	6.21 (0.59)	6.02 (2.75)	0.936
GSH (Umol/L)	4.91 (1.42)	2.86 (1.96)	4.49 (1.48)	2.61 (1.42)	0.732
Radiological classification					
Low					

(continued)

Table 5. *(continued)*

	≥60% FiO$_2$ oxygen therapy		Nasal 2–4 Lt/Dk oxygen therapy		*p*
	Mean (SD)	**Median (IQR)**	**Mean (SD)**	**Median (IQR)**	
MDA (nmol/mL)	5.65 (0.57)	5.73 (1.44)	5.49 (0.37)	5.50 (1.13)	0.917
GSH (Umol/L)	6.91 (4.11)	3.11 (6.92)	2.74 (0.22)	2.79 (0.98)	0.463
Intermediate					
MDA (nmol/mL)	8.04 (1.67)	7.30 (4.53)	6.98 (1.02)	6.83 (2.89)	0.263
GSH (Umol/L)	4.68 (1.51)	2.31 (6.35)	6.92 (3.42)	2.31 (7.10)	0.779
High					
MDA (nmol/mL)	6.33 (0.70)	5.52 (3.68)	6.41 (0.80)	5.38 (3.05)	0.717
GSH (Umol/L)	3.61 (0.53)	3.29 (1.76)	2.94 (0.30)	2.71 (1.40)	0.039

COPD, chronic obstructive pulmonary disease; FiO$_2$, the fraction of inspired O$_2$; GSH, glutathione; IQR, interquartile range; MDA, malondialdehyde; SD, standard deviation.

increasing their cells' susceptibility to the harmful effects of ROS.[39] Our study found that in patients with underlying DM, GSH levels, which were higher during oxygen therapy at FiO$_2$ ≥ 60%, decreased significantly during nasal cannula oxygen therapy at 2–4 L/m (*p* = 0.021). One explanation is that survivors may have utilized the potent antioxidant GSH for its protective effects during oxygen therapy at FiO$_2$ ≥ 60%; thus, GSH levels may have decreased after the patient recovered from the cytokine storm.

An infection with SARS-CoV-2 disrupts the redox balance, leading to increased protein oxidation and lipid peroxidation.[40] Yahaya *et al*.[41] studied 50 patients with COVID-19 and 21 healthy controls in a COVID-19 isolation center in Jigawa, Nigeria, to determine oxidant and antioxidant levels in COVID-19 infection. Vitamin C and E levels were found to be significantly lower in patients with COVID-19 compared to healthy controls, while vitamin A showed only a slight decrease in patients with COV-ID-19. GSH, catalase, and SOD levels were also lower in patients with COVID-19 than in controls. The plasma levels of these antioxidant trace elements were significantly lower in patients with COVID-19 compared to controls. When they assessed the plasma levels of oxidative stress markers PGF2α and MDA in patients with COVID-19 and controls, they found significantly higher levels of PGF2α in patients with COVID-19, though MDA levels were significantly lower in patients with COVID-19 compared to controls.[41] Another study examined plasma oxidative stress biomarkers in 115 hospitalized adult COVID-19 patients and found significant differences in levels of α-tocopherol, glutathione, superoxide dismutase, and advanced oxidation protein products between survivors and non-survivors. Crucially, glutathione levels below 327.2 µmol/mL were strongly linked to an increased risk of death, highlighting the critical role of oxidative stress in the progression and severity of COVID-19.[42] In our study, mean MDA levels were significantly higher in patients who died compared to those who survived. Although GSH levels were lower in patients who died than in survivors, the difference was not statistically significant. Additionally, in patients with severe radiological involvement, GSH levels, which were higher during oxygen therapy at FiO$_2$ ≥ 60%, decreased significantly after switching to nasal cannula oxygen therapy at 2–4 L/m (*p* = 0.039).

Among the limitations of our study, we acknowledge the relatively small sample size and the lack of control over other potential confounding factors, such as concurrent medications and the nutritional status of the patients.

Future directions

The findings from our study may have implications for personalizing oxygen therapy in hypertensive patients to minimize potential risks. Future studies should examine the long-term effects of high-flow oxygen therapy post-discharge in patients with respiratory failure, as this could further inform clinical practice.

Conclusions

We evaluated the potential of high-flow oxygen therapy to increase oxidative stress and inflammation in COVID-19 patients. Our observation of decreased MDA levels when transitioning hypertensive patients from high-flow to low-flow oxygen therapy was not observed in diabetic patients. In diabetic patients, GSH levels (a component of the antioxidant defense system) were higher during high-flow oxygen therapy, with a significant decrease noted when oxygen flow was reduced. The lack of impact on MDA levels when oxygen flow decreased in diabetic patients suggests that oxidative stress mechanisms may vary across different patient groups. Therefore, further research is needed to better understand the relationship between oxidative stress and oxygen therapy in patients with diabetes.

Conflict of interest

The authors declare no conflict of interest regarding the publication of this article.

Author contributions

Study concept and design (SÖ, PYG, MA, PMA), acquisition of the data (SÖ, PYG, MA,), assay performance and data analysis (PYG, MA, PMA), drafting of the manuscript (PYG, ŞY, PMA, NEY), and critical revision of the manuscript (PYG, MA, PMA).

All authors contributed equally to the writing or revision of the article and approved the final version of the manuscript.

Data sharing statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethical statement

This study was conducted in accordance with the ethical principles of the Declaration of Helsinki. Written informed consent was obtained from each patient prior to enrollment. The study protocol was approved by the Clinical Research Ethics Committee of Düzce University (decision no. 2020/275, dated January 4, 2021).This study was approved by the Clinical Research Ethics Committee of Düzce University (decision no. 2020/275, dated January 4, 2021.

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