Arch Clin Infect Dis. 2022 August; 17(4):e129799. https://doi.org/10.5812/archcid-129799.

Published online 2022 September 24. **Research Article**

The Efficacy of Methylprednisolone in Clinical Manifestations, Inflammatory Biomarkers, and Antioxidant Changes in the COVID-19 Patients

Atefeh Fakharian [1], Maryam Sadat Mirenayat [1,*], Fatemeh Ferdowsi [1], Seyed Bashir Mirtajani [2], Vajihozaman Khalili [3], Reyhaneh Zahiri [1] and Hamidreza Jamaati [1]

[1]Chronic Respiratory Diseases Research Center, National Research Institute of Tuberculosis and Lung Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2]Lung Transplantation Research Center, National Research Institute of Tuberculosis and Lung Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[3]Taha Teb, Clinical and Molecular Laboratory, Tehran, Iran

[*]*Corresponding author*: Chronic Respiratory Diseases Research Center, National Research Institute of Tuberculosis and Lung Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Email: maryammirenatyat2020@gmail.com

Received 2022 July 12; **Accepted** 2022 August 23.

Abstract

Background: The application of methylprednisolone in ARDS patients has led to a sustained reduction in inflammatory plasma cytokines and chemokines and has recently been used in the treatment of patients with SARS-CoV-2 infection.
Objectives: In this study, the effect of methylprednisolone on clinical symptoms and antioxidant changes of patients with COVID-19 has been investigated.
Methods: In the present study, patients with moderate to severe COVID-19 who required hospitalization were entered into the study phase. Then, in addition to standard treatment, patients received methylprednisolone at a dose of 250 mg intravenously over three days. Necessary evaluations include analysis of arterial blood gases, pulse oximetry, monitoring of patient clinical signs, examination of inflammatory biomarkers, and also receiving 10 cc of peripheral blood samples to check for antioxidant changes, at the beginning of the study, after 24 hours, and 72 hours after receiving methylprednisolone was on the agenda.
Results: Changes in fever, superoxide dismutase (SOD, Glutathione-S-Transferase (GST, the ferric reducing ability of plasma (FRAP, malondialdehyde (MDA, Nitric oxide, Ferritin, and TNF-α before treatment and 72 hours after treatment were significantly different between the two stages ($P < 0.05$).
Conclusions: The use of methylprednisolone improves the balance of antioxidants and immunological factors in patients with COVID-19 and thus improves some clinical indicators in these patients.

Keywords: COVID-19, Methylprednisolone, Antioxidant, Immunological Factors, Inflammatory Factors

1. Background

Our current world has undergone a huge transformation in various social, economic, and public health dimensions with the spread of the infectious agent called SARS-CoV-2. Coronavirus disease 2019 (COVID-19), in addition to taking the lives of countless people around the world, has threatened the survival of patients with a variety of consequences. Many patients with this infection show reduced symptoms, but some patients experience dysfunction of the various organs, which can be traced back to the physiological pathways associated with the clinical symptoms (1). After passing through the viral replication phase, SARS-CoV-2 induces widespread inflammatory responses in the host body by acting on immunological pathways (2). At this stage, phagocytic leukocytes such as neutrophils, eosinophils, monocytes, and macrophages stimulate the inflammatory mechanism by producing free radicals, mediating nuclear factor-kappa B (NF-κB) activation, and inducing transcription of cytokine-producing genes (3, 4). The proper functioning of the immune system depends on being safe from the harmful effects of overproduction of these compounds and, thus, the presence of a sufficient level of antioxidant defense (5).

Although definitive treatment for the infectious agent SARS-CoV-2 has not been reported, the treatment process is generally followed by the administration of immunosuppressive drugs, antiviral drugs, as well as anti-inflammatory drugs. Methylprednisolone is one of the anti-inflammatory compounds used to reduce the immune response in many diseases. This corticosteroid

is used in acute respiratory infections due to its anti-inflammatory effects, reduction of immune reactions, and improvement of clinical conditions (5). Studies have shown that the use of methylprednisolone in acute respiratory distress syndrome (ARDS) patients leads to a sustained decrease in inflammatory plasma cytokines and chemokines and also improves lung damage and multiple dysfunction syndromes (MODS). The use of methylprednisolone can also effectively reduce the time of mechanical ventilation and also mortality in the ICU (6). In addition, this drug has recently been used in the treatment of patients with SARS-CoV-2 infection (6).

2. Objectives

Considering the role of anti-inflammatory drugs in improving respiratory infections and considering the importance of antioxidant balance in controlling the inflammatory process, we investigated the effect of high dose methylprednisolone on clinical symptoms and antioxidant changes in patients with COVID-19.

3. Methods

The present study was performed as a non-randomized and non-blind comparative study with the ethical code IR.SBMU.NRITLD.REC.1399.227. Patients with COVID-19 (disease detection based on PCR and CT-SCAN molecular test) referred to Masih Daneshvari Hospital in Tehran from January to July 2021, who required hospitalization and were in moderate to severe disease. If they had inclusion criteria, they entered the study phase after obtaining written consent (predicted sample size: 30 patients). Inclusion criteria included evidence of new coronavirus (SARS-CoV-2) (clinical or paraclinical), saturation < 90, written consent of the study participants, age over 18 years, moderate to severe COVID-19 disease hospitalized, and in need of respiratory support. Exclusion criteria included pregnant or lactating patients, gastrointestinal bleeding, and a history of allergy to steroid drugs.

Then, in addition to standard treatment, patients received methylprednisolone at a dose of 250 mg intravenously over three days. Necessary evaluations, including arterial blood gas analysis, pulse oximetry, monitoring of patient clinical signs, and evaluation of inflammatory biomarkers, were performed. Also, receiving 10 cc of peripheral blood samples to study antioxidant changes was on the agenda at the beginning of the study, after 24 hours, and 72 hours after receiving methylprednisolone.

3.1. Statistical Analysis

In this study, quantitative variables were analyzed using the mean and standard deviation (SD), as well as qualitative variables using numbers (by mentioning percentages). To check the normality of quantitative variables, the Kolmogorov-Smirnov test was used using box diagrams. All the statistical tests used in this study were performed in two domains using SPSS 22 software, and the significance level was also considered to be 5%.

4. Results

Based on the results of this study, the population ratio was the same in patients, and according to Table 1, the average age of patients was 49.827 ± 12.803 years. This value was 52.40 ± 14.48 in the severe group of patients and 47.07 ± 10.56 in the moderate group. The numerical value of patients' weight also showed that although the mean weight of all patients was 78.250 ± 12.438 and had no difference in the two groups significantly (P = 0.543), this numerical value was higher in patients in the severe group with 79.71 ± 15.19 kg than the average group with 76.79 ± 9.27. Due to the approximately similar height of patients in the two groups, the BMI index was higher in patients with severe coronavirus than in the average group. Table 1 shows that these results are also true for the two indicators of the length of hospital stay and duration of hospitalization. Thus, in terms of the duration of illness to hospitalization, people in the severe group with an average of 10.40 ± 4.97 days went to treatment units significantly later (P = 0.006) compared to the moderate group with an average of 6.07 ± 2.84 days. However, the total mean time to hospitalization was 8.310 ± 4.575 in all patients.

According to Table 2, which examines the course of changes in patients' respiratory and clinical indices, the HR index in both groups was significantly reduced (P < 0.005). This change was significantly reduced for fever index (in patients with moderate severity) and Borg scale (in both groups) (P < 0.005). Although the course of changes in Spo2 and PaCO2 indices increased in the group of patients with a moderate form of the disease, the changes were not significant. However, the course of changes is constant in the two indicators of fever (in the group of patients with severe form) and RR. Also, urea, ALT, and bilirubin indices increased in both groups of patients and showed significant changes (P < 0.005). However, C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) had a significant decreasing trend in both groups (P < 0.005). It should be noted that the two indices of creatinine (Cr) in severe patients and LDH in moderate patients were significantly reduced (P < 0.005).

Examination of the antioxidant status of patients in the two groups treated with methylprednisolone showed that all antioxidants evaluated in this study underwent significant changes in both groups of patients (P < 0.005) (Table 3). Changes in the immunological factors of the

Table 1. Evaluation of Demographic Information of All Patients and Comparison of These Indicators Based on Two Groups of Patients with Severe and Moderate Forms of COVID-19

Groups	Total	Moderate, Mean ± SD	Severe, Mean ± SD	P-Value
Age	49.827 ± 12.803	47.07 ± 10.56	52.40 ± 14.48	0.270
Weight	78.250 ± 12.438	76.79 ± 9.27	79.71 ± 15.19	0.543
Height	167.071 ± 11.712	166.86 ± 10.85	167.29 ± 12.93	0.925
BMI	28.580 ± 4.611	28.59 ± 3.60	28.57 ± 5.53	0.992
Hospital stays	7.482 ± 2.429	6.86 ± 0.86	8.07 ± 3.22	0.769
The onset of symptoms is referred to	8.310 ± 4.575	6.07 ± 2.84	10.40 ± 4.97	0.006*

Abbreviation: BMI, body mass index.

patients showed that TNF-α significantly decreased in patients of both severe and moderate groups with COVID-19 after five days ($P < 0.001$). IL-10 increased significantly in both groups after five days ($P < 0.001$). Also, ferritin significantly decreased in both groups of severe and moderate patients after five days ($P < 0.001$ in the severe group and $P = 0.002$ in the moderate group) (Table 4).

According to Table 5, the changes in fever before treatment and 72 hours after treatment showed a significant difference between the two stages, so the fever decreased more in the moderate group. Changes in SOD before treatment and 72 hours after treatment were significantly different between the two stages, so there was a greater increase in the severe group. GST and FRAP changes before treatment and 72 hours after treatment were significantly different between the two stages, so there is a greater increase in the severe group. Changes in MDA before treatment and 72 hours after treatment indicated a significant difference between the two stages, so there was a decreasing trend in the severe group, whereas there was an increasing trend in the moderate group. Changes in TNF-α before treatment and 72 hours after treatment showed a significant difference between the two stages, so a greater reduction was observed in the severe group. The changes in ferritin before treatment and 72 hours after treatment between the two stages were significantly different, so there was a greater decrease in the severe group. Nitric oxide changes before treatment and 72 hours after treatment were significantly different between the two stages, so there was more increase in the severe group.

5. Discussion

This study aimed to evaluate the efficacy of methylprednisolone on clinical manifestations, inflammatory biomarkers, and antioxidant changes in patients with COVID-19. We hypothesized that this drug could improve pulmonary function, shortness of breath, immunological markers, and antioxidant markers in these patients. The main findings of the study showed that the immunological and antioxidant parameters of the patients were significantly improved, which confirms our hypothesis.

Based on the results obtained from past studies, infection with SARS-CoV-2 destroys lung tissue cells and stimulation of immune response. This immune response recruits innate immune cells, such as macrophages and monocytes, as the primary response, then T and B cells (adaptive immune cells) are activated against the infection (7). Though in many patients, this primary immune response can effectively stop the infection, in a situation where the patient's immune system creates a disturbed immune response, a cytokine storm and subsequently pneumonia is created. Investigations have shown that the mortality and severity of COVID-19 are influenced by the increase in the concentration of inflammatory factors such as ferritin, CRP, and cytokine storm created with interleukins, such as TNF-α and Monocyte Chemoattractant Protein-1 (MCP-1). These cytokines can increase the ratio of neutrophils to lymphocytes (8). Infection with SARS-CoV-2 causes the activation of NOD-like receptor protein 3 (NLRP3), as a pattern recognition receptor (PRR), which is responsible for recognition of damage-associated molecular patterns (DAMPs) or pathogen-associated molecular patterns (PAMPs). The PAMPs are involved in the recruitment and clustering of multi-protein complexes called inflammasomes (9). Inflammation due to NLRP3 activation eventually causes cell death, known as pyroptosis and apoptosis (10). Therefore, the progression of lung damage is often influenced by the activity of type II alveolar cells, endothelial cells, and the activation of the innate immune responses (11). With the activity of alveolar macrophages, the cytokine storm is initiated that stimulates endothelial cells, platelets, and neutrophils, and thus a collection of platelets and neutrophils is created on the surface of endothelial cells (12-18). This isolation of these neutrophilic and platelet structures from pulmonary arteries causes immunothrombosis (19). Convincing evidence suggests that immunothrombosis is a major determinant of the production of microthrombi and microemboli in the capillaries of alveoli circulation,

Table 4. Evaluation of Changes in Immunological Indices in Patients and Comparison of These Changes in Two Groups of Patients with a Severe and Moderate Form

	Mean ± SD	Percentiles			P-Value
		25th	50th	75th	
TNF-α					
Moderate					< 0.001
Day 1	20.667 ± 0.894	20.160	20.455	21.172	
Day 2	18.535 ± 0.816	17.775	18.330	19.550	
Day 5	17.379 ± 0.889	16.600	17.075	18.310	
Severe					< 0.001
Day 1	38.423 ± 6.597	32.500	41.870	43.440	
Day 2	29.764 ± 5.662	24.500	30.810	34.500	
Day 5	18.666 ± 0.443	18.400	18.510	19.080	
IL-10					
Moderate					< 0.001
Day 1	13.684 ± 0.394	13.3225	13.6400	14.1125	
Day 2	11.890 ± 0.435	11.5025	12.0000	12.2475	
Day 5	14.255 ± 0.563	13.7850	14.2900	14.6025	
Severe					< 0.001
Day 1	15.713 ± 0.905	15.4400	15.8300	16.3200	
Day 2	13.872 ± 0.760	13.4700	13.8800	14.2300	
Day 5	16.050 ± 0.670	15.7000	15.8400	16.5200	
Ferritin					
Moderate					0.002
Day 1	74.214 ± 13.75	63.000	67.000	88.750	
Day 2	74.142 ± 12.94	64.500	67.500	88.750	
Day 5	72.214 ± 13.43	61.750	67.000	86.750	
Severe					< 0.001
Day 1	246.13 ± 47.25	248.000	258.000	264.000	
Day 2	126.73 ± 21.04	113.000	122.000	137.000	
Day 5	97.266 ± 7.676	89.000	99.000	101.000	

formation of fibrin deposits, and sometimes spread intravascular clot production (20). Therefore, increasing the concentration of neutrophils in the interstitial tissue of the lung and alveoli plays a significant role in creating a cytokine storm and tissue damage, leading to the deterioration of the patient's clinical conditions and ARDS (21).

Therefore, tissue damage caused by SARS-CoV-2 infection (especially lung tissue) is affected by several mechanisms and factors. However, studies have shown that Reactive oxygen species (ROS) is one of the factors that play a pivotal role in the initiation and progression of these inflammatory mechanisms (22). Another effective factor in causing inflammation is NLRP3 (23). However, other pathological pathways may be involved in the induction of NLRP3. The development of this inflammation caused by NLRP3 is influenced by the expression of IL-18 and IL-1β due to the stimulation of NF-κB (24). Indeed, when the innate response fails to control infection, NLRP3 overactivity leads to mitochondrial dysfunction, DAMPs release, and increased pyroptosis, leading to virus spread and extensive destruction of damaged tissues (25).

Today, many antiviral treatments are based on the effect on intracellular redox pathways. Studies have shown that respiratory viral infections, especially SARS-CoV-2, inhibit nuclear factor erythroid 2-related factor 2 (NRF2) and activate nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathways despite disrupting ROS production, leading to inflammation and oxidative dam-

Table 5. Comparison of Clinical and Immunological Indicators of Patients in Two Moderate and Severe Groups

Groups	Moderate, Mean ± SD	Severe, Mean ± SD	P-Value
HR. diff	-16.21 ± 13.16	-24.87 ± 25.92	0.238
RR. diff	0.71 ± 1.49	-0.47 ± 1.73	0.068
Fever. diff	-0.26 ± 0.26	-0.11 ± 0.56	0.0028*
Borg scale. diff	-1.86 ± 1.10	-1.93 ± 2.05	0.717
SpO2.diff	0.57 ± 2.34	1.07 ± 4.36	0.330
PaCO2. diff	6.45 ± 19.68	-0.13 ± 9.46	0.144
WBC. diff	1.99 ± 3.52	1.93 ± 8.64	0.462
Urea. diff	13.86 ± 14.94	19.60 ± 21.15	0.948
Cr. diff	-0.09 ± 0.23	-0.45 ± 1.00	0.205
AST. diff	10.00 ± 18.95	9.13 ± 18.08	0.983
ALT. diff	33.00 ± 51.13	31.00 ± 39.75	0.896
ALP. diff	-5.29 ± 24.33	-21.40 ± 47.06	0.485
Bilirubin. diff	0.04 ± 0.32	-0.02 ± 0.28	0.551
CRP. diff	-36.08 ± 32.38	-30.36 ± 26.54	0.152
LDH. diff	-88.50 ± 80.65	-53.93 ± 171.35	0.793
ESR. diff	-19.21 ± 19.13	-28.27 ± 24.21	0.315
SOD. diff	0.11 ± 0.36	7.58 ± 27.67	0.003*
GPX. diff	0.24 ± 0.59	0.28 ± 0.34	0.647
GST. diff	0.06 ± 0.04	0.33 ± 0.10	< 0.001
FRAP. diff	2.13 ± 9.05	19.61 ± 9.31	< 0.001
MDA. diff	0.44 ± 0.35	-0.11 ± 0.49	0.002*
Albumin. diff	-0.95 ± 0.49	-0.64 ± 0.34	0.093
Zn. diff	1.64 ± 3.61	0.40 ± 3.40	0.825
TNF-α. diff	-3.29 ± 1.28	-19.76 ± 6.64	< 0.001
IL-10. diff	0.57 ± 0.51	0.34 ± 0.72	0.238
Ferritin. diff	-2.00 ± 2.96	-148.87 ± 43.87	< 0.001
Nitric oxide. diff	-0.05 ± 0.60	1.05 ± 1.06	0.003*

age. Therefore, examining NRF2 activators in patients with COVID-19 may be of importance. The clinical benefits of dexamethasone, hydrocortisone, or methylprednisolone have been evaluated in previous studies. The review of these studies showed that the period of treatment with corticosteroids ranged from three to 14 days, and the dosage of the drug was gradually increased. The effectiveness of dexamethasone compared to methylprednisolone showed different results (26). Also, the clinical conditions of the patients in different studies showed various changes and might have been affected by factors such as the severity of the disease, the type of corticosteroid, the dosage, and the statistical power of the study (27).

The results of studies on the effectiveness of methylprednisolone have not directly indicated the positive effects of this drug. A study evaluated methylprednisolone by examining 393 patients with COVID-19 at a dose of 1 mg/kg compared with placebo. The result of this study confirmed the positive role of methylprednisolone on 28-day mortality of patients. However, it did not affect virus clearance (28). In another study conducted on 85 patients with moderate to severe COVID-19, the patients received 40 mg of methylprednisolone for three days, and 20 mg was given to the patients three days later. The researchers found that methylprednisolone could reduce the mortality and severity of the disease in these patients (29). Also, a quasi-experimental study of patients with moderate to severe COVID-19 who used 0.5 - 1 mg of methylprednisolone for three days confirmed the effect of this drug on reducing mortality compared to the control group in which the

patients were transferred to the ICU (30). The timing of the use of corticosteroids as a key factor in the treatment of the onset of shortness of breath was also supported. These measures appeared to prevent the progression of the disease associated with the host pro-inflammatory responses. On the other hand, a retrospective cohort study that examined 205 patients with severe phases of COVID-19 showed that taking 80 mg of methylprednisolone daily did not significantly change the mortality of patients (31).

Although observational and randomized trial data generally support the role of corticosteroids in the treatment of severe COVID-19, most studies have linked this advantage to the need for respiratory support (32). In addition, examining the progress of the disease in different people has shown that different clinical responses and clinical conditions of each patient can affect the type of reaction to corticosteroids. Thus, the benefits of corticosteroid therapy may also depend on the degree of inflammation (33). The present study has limitations, such as being monocentric with observational and retrospective nature. However, this study was unique to the patient population in Iran because it focused on a subset of patients developing severe inflammatory syndrome. According to the present information, no study has compared the effect of methylprednisolone treatment based on the degree of severity on immunological and antioxidant indicators. The results obtained from our study show that the effectiveness of methylprednisolone can be beyond what has been discussed in other studies.

5.1. Conclusions

The use of methylprednisolone by improving the balance of antioxidants and immunological factors in patients with COVID-19 improves some clinical indicators in these patients. Thus, methylprednisolone can be considered a drug of choice in patients with moderate to severe COVID-19.

Acknowledgments

The authors thank the staff of the internal medicine and rehabilitation department of Masih Daneshvari Hospital in Tehran, Iran, and all those who participated in this project.

Footnotes

Authors' Contribution: Study concept and design: A. F., H. J., and S. B. M.; Analysis and interpretation of data: MS. M., and V. KH.; Drafting of the manuscript: R. Z. and F. F.; Critical revision of the manuscript for important intellectual content: A. F., H. J., and MS. M.; Statistical analysis: R. Z. and S. B. M.

Conflict of Interests: Funding or Research support: Shahid Beheshti University of medical science. Employment: Chronic Respiratory Diseases Research Center (CRDRC), National Research Institute of Tuberculosis and Lung Diseases (NRITLD). The current research was done as a thesis in the fellowship stage. Personal financial interests: The budget of the project was provided from internal sources of Shahid Beheshti University of Medical Sciences. Stocks or shares in companies: Laboratory tests were performed without the participation of companies and in the laboratory of Masih Deneshvari Hospital in Tehran, Iran. Consultation fees: In this research, there was no need for consultation and its cost. Patents: No patents have been made in this research. Personal or professional relations with organizations and individuals (parents and children, wife and husband, family relationships, etc.): There are not. Unpaid membership in a government or non-governmental organization: The contributing authors in this research are professors of Shahid Beheshti University of Medical Sciences, pulmonary fellowship students and researchers at Masih Daneshvari Hospital in Tehran, Iran, and there is no family relationship between these people and organizations and companies. Are you one of the editorial board members or a reviewer of this journal? No.

Ethical Approval: This study is approved under the ethical approval code of IR.SBMU.NRITLD.REC.1399.227 (link: ethics.research.ac.ir/EthicsProposalView.php?id=185695).

Funding/Support: The entire budget of this project was provided by Shahid Beheshti University of Medical Sciences.

Informed Consent: Written informed consent was obtained from participants.

References

1. Robba C, Battaglini D, Pelosi P, Rocco PRM. Multiple organ dysfunction in SARS-CoV-2: MODS-CoV-2. *Expert Rev Respir Med*. 2020;**14**(9):865–8. [PubMed ID: 32567404]. [PubMed Central ID: PMC7441756]. https://doi.org/10.1080/17476348.2020.1778470.
2. Mansourabadi AH, Sadeghalvad M, Mohammadi-Motlagh HR, Rezaei N. The immune system as a target for therapy of SARS-CoV-2: A systematic review of the current immunotherapies for COVID-19. *Life Sci*. 2020;**258**:118185. [PubMed ID: 32750438]. [PubMed Central ID: PMC7395832]. https://doi.org/10.1016/j.lfs.2020.118185.
3. Carcaterra M, Caruso C. Alveolar epithelial cell type II as main target of SARS-CoV-2 virus and COVID-19 development via NF-Kb pathway deregulation: A physio-pathological theory. *Med Hypotheses*. 2021;**146**:110412. [PubMed ID: 33308936]. [PubMed Central ID: PMC7681037]. https://doi.org/10.1016/j.mehy.2020.110412.
4. Berger MM, Chiolero RL. Antioxidant supplementation in sepsis and systemic inflammatory response syndrome. *Crit Care Med*. 2007;**35**(9 Suppl):S584–90. [PubMed ID: 17713413]. https://doi.org/10.1097/01.CCM.0000279189.81529.C4.
5. Stern A, Skalsky K, Avni T, Carrara E, Leibovici L, Paul M. Corticosteroids for pneumonia. *Cochrane Database Syst Rev*. 2017;**12**. CD007720. [PubMed ID: 29236286]. [PubMed Central ID: PMC6486210]. https://doi.org/10.1002/14651858.CD007720.pub3.

6. Drago BB, Kimura D, Rovnaghi CR, Schwingshackl A, Rayburn M, Meduri GU, et al. Double-blind, placebo-controlled pilot randomized trial of methylprednisolone infusion in pediatric acute respiratory distress syndrome. *Pediatr Crit Care Med*. 2015;**16**(3):e74–81. [PubMed ID: 25634565]. https://doi.org/10.1097/PCC.0000000000000349.
7. Beacon TH, Su RC, Lakowski TM, Delcuve GP, Davie JR. SARS-CoV-2 multifaceted interaction with the human host. Part II: Innate immunity response, immunopathology, and epigenetics. *IUBMB Life*. 2020;**72**(11):2331–54. [PubMed ID: 32936531]. https://doi.org/10.1002/iub.2379.
8. Matthay MA, Luetkemeyer AF. IL-6 Receptor Antagonist Therapy for Patients Hospitalized for COVID-19: Who, When, and How? *JAMA*. 2021;**326**(6):483–5. [PubMed ID: 34228779]. https://doi.org/10.1001/jama.2021.11121.
9. Ko JJ, Wu C, Mehta N, Wald-Dickler N, Yang W, Qiao R. A Comparison of Methylprednisolone and Dexamethasone in Intensive Care Patients With COVID-19. *J Intensive Care Med*. 2021;**36**(6):673–80. [PubMed ID: 33632000]. https://doi.org/10.1177/0885066621994057.
10. Majmundar M, Kansara T, Lenik JM, Park H, Ghosh K, Doshi R, et al. Efficacy of corticosteroids in non-intensive care unit patients with COVID-19 pneumonia from the New York Metropolitan region. *PLoS One*. 2020;**15**(9). e0238827. [PubMed ID: 32903258]. [PubMed Central ID: PMC7480842]. https://doi.org/10.1371/journal.pone.0238827.
11. Saleki K, Banazadeh M, Miri NS, Azadmehr A. Triangle of cytokine storm, central nervous system involvement, and viral infection in COVID-19: the role of sFasL and neuropilin-1. *Rev Neurosci*. 2022;**33**(2):147–60. [PubMed ID: 34225390]. https://doi.org/10.1515/revneuro-2021-0047.
12. Dan Dunn J, Alvarez LA, Zhang X, Soldati T. Reactive oxygen species and mitochondria: A nexus of cellular homeostasis. *Redox Biol*. 2015;**6**:472–85. [PubMed ID: 26432659]. [PubMed Central ID: PMC4596921]. https://doi.org/10.1016/j.redox.2015.09.005.
13. Anand SK, Tikoo SK. Viruses as modulators of mitochondrial functions. *Adv Virol*. 2013;**2013**:738794. [PubMed ID: 24260034]. [PubMed Central ID: PMC3821892]. https://doi.org/10.1155/2013/738794.
14. de Las Heras N, Martin Gimenez VM, Ferder L, Manucha W, Lahera V. Implications of Oxidative Stress and Potential Role of Mitochondrial Dysfunction in COVID-19: Therapeutic Effects of Vitamin D. *Antioxidants (Basel)*. 2020;**9**(9). [PubMed ID: 32967329]. [PubMed Central ID: PMC7555731]. https://doi.org/10.3390/antiox9090897.
15. Burtscher J, Cappellano G, Omori A, Koshiba T, Millet GP. Mitochondria: In the Cross Fire of SARS-CoV-2 and Immunity. *iScience*. 2020;**23**(10):101631. [PubMed ID: 33015593]. [PubMed Central ID: PMC7524535]. https://doi.org/10.1016/j.isci.2020.101631.
16. Shi CS, Qi HY, Boularan C, Huang NN, Abu-Asab M, Shelhamer JH, et al. SARS-coronavirus open reading frame-9b suppresses innate immunity by targeting mitochondria and the MAVS/TRAF3/TRAF6 signalosome. *J Immunol*. 2014;**193**(6):3080–9. [PubMed ID: 25135833]. [PubMed Central ID: PMC4179872]. https://doi.org/10.4049/jimmunol.1303196.
17. McBride R, Fielding BC. The role of severe acute respiratory syndrome (SARS)-coronavirus accessory proteins in virus pathogenesis. *Viruses*. 2012;**4**(11):2902–23. [PubMed ID: 23202509]. [PubMed Central ID: PMC3509677]. https://doi.org/10.3390/v4112902.
18. Scherz-Shouval R, Elazar Z. Regulation of autophagy by ROS: physiology and pathology. *Trends Biochem Sci*. 2011;**36**(1):30–8. [PubMed ID: 20728362]. https://doi.org/10.1016/j.tibs.2010.07.007.
19. Gordon DE, Jang GM, Bouhaddou M, Xu J, Obernier K, White KM, et al. A SARS-CoV-2 protein interaction map reveals targets for drug repurposing. *Nature*. 2020;**583**(7816):459–68. [PubMed ID: 32353859]. [PubMed Central ID: PMC7431030]. https://doi.org/10.1038/s41586-020-2286-9.
20. Filadi R, Leal NS, Schreiner B, Rossi A, Dentoni G, Pinho CM, et al. TOM70 Sustains Cell Bioenergetics by Promoting IP3R3-Mediated ER to Mitochondria Ca(2+) Transfer. *Curr Biol*. 2018;**28**(3):369–382 e6. [PubMed ID: 29395920]. https://doi.org/10.1016/j.cub.2017.12.047.
21. Silvagno F, Vernone A, Pescarmona GP. The Role of Glutathione in Protecting against the Severe Inflammatory Response Triggered by COVID-19. *Antioxidants (Basel)*. 2020;**9**(7). [PubMed ID: 32708578]. [PubMed Central ID: PMC7402141]. https://doi.org/10.3390/antiox9070624.
22. Wu C, Chen X, Cai Y, Xia J, Zhou X, Xu S, et al. Risk Factors Associated With Acute Respiratory Distress Syndrome and Death in Patients With Coronavirus Disease 2019 Pneumonia in Wuhan, China. *JAMA Intern Med*. 2020;**180**(7):934–43. [PubMed ID: 32167524]. [PubMed Central ID: PMC7070509]. https://doi.org/10.1001/jamainternmed.2020.0994.
23. Gong J, Dong H, Xia QS, Huang ZY, Wang DK, Zhao Y, et al. Correlation analysis between disease severity and inflammation-related parameters in patients with COVID-19 pneumonia. *medRxiv*. 2020. [PubMed ID: 33349241]. [PubMed Central ID: PMC7750784]. https://doi.org/10.1186/s12879-020-05681-5.
24. Martinon F. Detection of immune danger signals by NALP3. *J Leukoc Biol*. 2008;**83**(3):507–11. [PubMed ID: 17982111]. https://doi.org/10.1189/jlb.0607362.
25. Tian S, Hu W, Niu L, Liu H, Xu H, Xiao SY. Pulmonary Pathology of Early-Phase 2019 Novel Coronavirus (COVID-19) Pneumonia in Two Patients With Lung Cancer. *J Thorac Oncol*. 2020;**15**(5):700–4. [PubMed ID: 32114094]. [PubMed Central ID: PMC7128866]. https://doi.org/10.1016/j.jtho.2020.02.010.
26. Fink SL, Cookson BT. Apoptosis, pyroptosis, and necrosis: mechanistic description of dead and dying eukaryotic cells. *Infect Immun*. 2005;**73**(4):1907–16. [PubMed ID: 15784530]. [PubMed Central ID: PMC1087413]. https://doi.org/10.1128/IAI.73.4.1907-1916.2005.
27. Faust H, Mangalmurti NS. Collateral damage: necroptosis in the development of lung injury. *Am J Physiol Lung Cell Mol Physiol*. 2020;**318**(2):L215–25. [PubMed ID: 31774305]. [PubMed Central ID: PMC7052671]. https://doi.org/10.1152/ajplung.00065.2019.
28. Mason RJ. Pathogenesis of COVID-19 from a cell biology perspective. *Eur Respir J*. 2020;**55**(4). [PubMed ID: 32269085]. [PubMed Central ID: PMC7144260]. https://doi.org/10.1183/13993003.00607-2020.
29. Frantzeskaki F, Armaganidis A, Orfanos SE. Immunothrombosis in Acute Respiratory Distress Syndrome: Cross Talks between Inflammation and Coagulation. *Respiration*. 2017;**93**(3):212–25. [PubMed ID: 27997925]. https://doi.org/10.1159/000453002.
30. El Haouari M. Platelet Oxidative Stress and its Relationship with Cardiovascular Diseases in Type 2 Diabetes Mellitus Patients. *Curr Med Chem*. 2019;**26**(22):4145–65. [PubMed ID: 28982316]. https://doi.org/10.2174/0929867324666171005114456.
31. Freedman JE. Oxidative stress and platelets. *Arterioscler Thromb Vasc Biol*. 2008;**28**(3):s11–6. [PubMed ID: 18174453]. https://doi.org/10.1161/ATVBAHA.107.159178.
32. Oynebraten I, Barois N, Bergeland T, Kuchler AM, Bakke O, Haraldsen G. Oligomerized, filamentous surface presentation of RANTES/CCL5 on vascular endothelial cells. *Sci Rep*. 2015;**5**:9261. [PubMed ID: 25791723]. [PubMed Central ID: PMC4367157]. https://doi.org/10.1038/srep09261.
33. Mirenayat MS, Abedini A, Kiani A, Eslaminejad A, Adimi Naghan P, Malekmohammad M, et al. National Research Institute of Tuberculosis and Lung Disease (NRITLD) Protocol for the Treatment of Patients with COVID-19. *Iran J Pharm Res*. 2022;**21**(1). e123947. [PubMed ID: 35765502]. [PubMed Central ID: PMC9191225]. https://doi.org/10.5812/ijpr.123947.

Table 2. Evaluation of Changes in Clinical and Biochemical Indicators in the Two Groups of Patients with Severe and Moderate Forms of COVID-19

	Mean ± SD	Percentiles			P-Value
		25th	50th	75th	
HR					
Moderate					0.006
Before	95.2143 ± 14.159	83.750	95.500	108.500	
After 24 h	82.0000 ± 14.071	73.750	78.000	89.2500	
After 72 h	79.0000 ± 8.3757	72.200	80.500	85.2500	
Severe					0.004
Before	107.1333 ± 26.51	94.000	110.00	120.000	
After 24 h	86.6000 ± 7.3949	80.00	85.000	93.0000	
After 72 h	82.2667 ± 5.7004	77.000	82.000	86.0000	
RR					
Moderate					0.078
Before	17.8571 ± 0.9492	18.000	18.000	18.0000	
After 24 h	18.8571 ± 1.5118	18.000	18.000	20.0000	
After 72 h	18.5714 ± 1.2225	18.000	18.000	18.5000	
Severe					0.439
Before	19.1333 ± 1.2459	18.000	19.000	20.0000	
After 24 h	18.8667 ± 1.6417	18.000	18.000	20.0000	
After 72 h	18.6667 ± 0.9759	18.000	18.000	20.0000	
Fever					
Moderate					0.017
Before	36.7786 ± 0.2778	36.500	36.700	37.0000	
After 24 h	36.6286 ± 0.2701	36.500	36.550	36.9250	
After 72 h	36.5143 ± 0.1657	36.500	36.500	36.6000	
Severe					0.853
Before	36.7929 ± 0.5239	36.500	36.650	36.8500	
After 24 h	36.6571 ± 0.3081	36.500	36.600	37.0000	
After 72 h	36.6500 ± 0.1605	36.500	36.600	36.7250	
Borg scale					
Moderate					0.003
Before	5.3636 ± 2.06265	4.0000	5.0000	8.0000	
After 24 h	4.7273 ± 1.90215	3.0000	4.0000	7.0000	
After 72 h	3.5455 ± 1.43970	2.0000	3.0000	5.0000	
Severe					0.006
Before	4.4545 ± 2.54416	3.0000	5.0000	5.0000	
After 24 h	3.3636 ± 1.68954	2.0000	3.0000	5.0000	
After 72 h	2.7273 ± 1.95402	2.0000	2.0000	5.0000	
SpO_2					
Moderate					0.502
Before	94.7143 ± 1.89852	93.000	95.000	96.0000	

After 24 h	94.6429 ± 1.44686	93.000	94.500	96.0000	
After 72 h	95.2857 ± 1.85757	94.750	95.500	96.0000	
Severe					0.225
Before	93.0667 ± 2.89005	90.000	94.000	96.0000	
After 24 h	94.3333 ± 1.83874	93.000	95.000	96.0000	
After 72 h	91.8267 ± 8.74524	92.000	94.000	96.0000	
$PaCO_2$					
Moderate					0.212
Before	47.100 ± 10.5014	39.500	49.000	51.500	
After 24 h	48.169 ± 5.67104	42.900	47.000	54.500	
After 72 h	52.430 ± 13.3051	44.500	52.000	62.500	
Severe					0.982
Before	50.357 ± 5.95751	46.500	51.000	53.500	
After 24 h	47.628 ± 8.72683	40.750	46.350	53.000	
After 72 h	50.435 ± 8.18795	45.750	51.100	57.650	
WBC					
Moderate					0.0397
Before	7.333 ± 3.79006	4.5000	6.4000	10.100	
After 24 h	8.250 ± 3.38369	5.2650	8.2000	10.475	
After 72 h	9.293 ± 3.16487	6.7100	10.4900	11.500	
Severe					0.789
Before	14.911 ± 22.5218	5.7475	8.7500	11.125	
After 24 h	9.155 ± 4.44306	4.9625	8.4700	12.207	
After 72 h	11.241 ± 7.45625	6.1625	8.5700	13.617	
Urea					
Moderate					0.004
Before	30.571 ± 12.768	21.0000	24.500	42.250	
After 24 h	37.000 ± 15.491	28.5000	33.500	42.250	
After 72 h	44.428 ± 10.278	37.7500	46.000	53.000	
Severe					0.001
Before	33.400 ± 13.589	28.0000	32.000	41.000	
After 24 h	42.266 ± 18.926	33.0000	37.000	44.000	
After 72 h	53.000 ± 28.886	38.0000	45.000	55.000	
Cr					
Moderate					0.066
Before	1.078 ± 0.196	0.900	1.100	1.200	
After 24 h	1.014 ± 0.210	0.800	1.000	1.125	
After 72 h	0.985 ± 0.313	0.875	1.050	1.200	
Severe					0.001
Before	1.726 ± 2.107	1.100	1.100	1.300	
After 24 h	1.320 ± 1.057	1.000	1.100	1.200	
After 72 h	1.280 ± 1.173	1.000	1.100	1.200	
AST					
Moderate					0.257

Before	33.714 ± 11.179	26.750	32.000	40.250	
After 24 h	39.714 ± 10.313	31.500	37.500	47.500	
After 72h	43.714 ± 20.693	24.750	37.000	55.000	
Severe					0.138
Before	37.142 ± 16.737	24.500	34.500	47.500	
After 24 h	36.142 ± 13.960	23.750	34.500	49.250	
After 72h	43.357 ± 21.168	25.750	39.500	50.750	
ALT					
Moderate					0.017
Before	36.857 ± 17.064	24.000	35.500	48.250	
After 24 h	41.000 ± 14.889	36.000	40.500	44.750	
After 72 h	69.857 ± 49.155	47.000	56.000	61.750	
Severe					0.008
Before	36.133 ± 23.008	17.000	32.000	46.000	
After 24 h	34.400 ± 15.701	17.000	33.000	53.000	
After 72 h	67.133 ± 42.914	27.000	65.000	85.000	
ALP					
Moderate					0.395
Before	142.000 ± 40.876	102.250	148.000	173.50	
After 24 h	164.285 ± 118.781	97.750	141.500	153.00	
After 72 h	136.714 ± 31.699	105.250	135.500	164.50	
Severe					0.124
Before	191.733 ± 151.732	93.000	124.000	251.00	
After 24 h	204.000 ± 194.579	113.000	133.000	210.00	
After 72 h	170.333 ± 121.917	111.000	145.000	153.00	
Bil					
Moderate					0.031
Before	0.669 ± 0.375	0.400	0.600	0.900	
After 24 h	0.515 ± 0.293	0.300	0.500	0.650	
After 72 h	0.715 ± 0.237	0.550	0.700	0.900	
Severe					0.014
Before	0.666 ± 0.179	0.500	0.700	0.800	
After 24 h	0.480 ± 0.169	0.400	0.400	0.600	
After 72 h	0.646 ± 0.289	0.400	0.700	0.900	
CRP					
Moderate					0.002
Before	50.583 ± 21.334	41.250	44.000	60.250	
After 24 h	41.416 ± 24.254	23.000	39.000	49.000	
After 72 h	15.833 ± 25.711	3.250	8.500	14.250	
Severe					< 0.001
Before	54.357 ± 22.245	41.750	54.500	60.250	
After 24 h	71.071 ± 24.627	47.750	64.000	96.500	
After 72 h	24.000 ± 12.428	13.750	22.000	30.500	
LDH					

Moderate					< 0.001
Before	516.285 ± 96.497	448.750	493.000	595.000	
After 24 h	506.142 ± 124.445	419.000	503.500	559.250	
After 72 h	427.785 ± 88.105	373.500	432.000	460.500	
Severe					0.091
Before	545.466 ± 192.425	392.000	506.000	749.000	
After 24 h	518.400 ± 148.267	375.000	532.000	627.000	
After 72 h	491.533 ± 171.835	375.000	404.000	659.000	
ESR					
Moderate					< 0.001
Before	45.153 ± 22.575	28.500	50.000	64.000	
After 24 h	54.461 ± 28.215	24.500	61.000	75.500	
After 72 h	25.615 ± 17.153	7.500	24.000	41.000	
Severe					0.001
Before	53.000 ± 22.934	36.250	47.500	74.000	
After24 h	50.285 ± 21.620	37.500	49.000	64.500	
After 72 h	22.357 ± 12.743	12.750	20.000	31.500	

Abbreviations: HR, heart rate; RR, respiratory rate.

Table 3. Evaluation of the Status of Changes in Antioxidant Indices in Patients and Comparison of These Changes in Two Groups of Patients with Severe and Moderate Forms of COVID-19

	Mean ± SD	Percentiles			P-Value
		25th	50th	75th	
SOD					
Moderate					0.023
Day 1	2.128 ± 0.140	1.970	2.195	2.242	
Day 2	2.419 ± 0.293	2.200	2.270	2.762	
Day 5	2.236 ± 0.360	2.087	2.305	2.495	
Severe					< 0.001
Day 1	1.563 ± 0.189	1.430	1.550	1.600	
Day 2	1.962 ± 0.091	1.900	1.930	2.010	
Day 5	9.140 ± 27.625	1.920	1.980	2.110	
GPX					
Moderate					0.708
Day 1	19.717 ± 0.404	19.470	19.820	20.087	
Day 2	19.814 ± 0.345	19.717	19.830	19.947	
Day 5	19.953 ± 0.431	19.807	19.930	20.185	
Severe					0.038
Day 1	18.802 ± 0.630	18.170	18.880	19.230	
Day 2	18.943 ± 0.750	18.200	18.970	19.500	
Day 5	19.082 ± 0.645	18.410	19.270	19.660	
GST					
Moderate					0.003
Day 1	0.882 ± 0.036	0.865	0.890	0.910	
Day 2	0.902 ± 0.053	0.855	0.895	0.952	
Day	0.939 ± 0.035	0.907	0.940	0.970	
Severe					< 0.001
Day 1	0.567 ± 0.106	0.520	0.540	0.570	
Day 2	0.628 ± 0.098	0.580	0.600	0.640	
Day 5	0.897 ± 0.037	0.870	0.900	0.920	
FRAP					
Moderate					0.330
Day 1	616.73 ± 8.538	613.750	618.70	621.050	
Day 2	618.10 ± 6.387	614.602	619.80	622.300	
Day 5	618.85 ± 3.463	616.100	619.05	621.117	
Severe					< 0.001
Day 1	598.36 ± 9.175	590.940	598.80	603.480	
Day 2	617.72 ± 3.763	617.800	619.24	620.000	
Day 5	617.98 ± 3.222	617.090	619.44	620.090	
ALB					
Moderate					< 0.001
Day 1	4.799 ± 0.337	4.495	4.705	5.147	

Day 2	4.230 ± 0.377	4.017	4.240	4.350	
Day 5	3.850 ± 0.353	3.572	3.885	4.205	
Severe					< 0.001
Day 1	4.240 ± 0.188	4.120	4.220	4.330	
Day 2	3.908 ± 0.197	3.800	3.910	4.110	
Day 5	3.599 ± 0.274	3.410	3.570	3.740	
Zn					
Moderate					0.437
Day 1	65.285 ± 3.383	63.000	65.000	67.000	
Day 2	64.857 ± 3.840	62.750	65.000	67.000	
Day 5	66.928 ± 2.644	65.000	66.000	69.000	
Severe					0.030
Day 1	61.200 ± 6.731	55.000	62.000	67.000	
Day 2	60.733 ± 6.430	54.000	62.000	66.000	
Day 5	61.600 ± 7.058	54.000	63.000	66.000	
NO					
Moderate					0.810
Day 1	22.691 ± 0.520	22.465	22.640	23.107	
Day 2	22.820 ± 0.567	22.407	22.875	23.135	
Day 5	22.640 ± 0.513	22.370	22.630	23.100	
Severe					0.010
Day 1	21.148 ± 1.130	20.020	21.120	21.980	
Day 2	21.950 ± 0.802	21.700	22.010	22.270	
Day 5	22.202 ± 0.472	21.740	22.190	22.480	

Abbreviations: GPX, glutathione peroxidase; GST, glutathione-s-transferase.