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Nuclear Factor Erythroid 2-Related Factor (NRF2), Heme Oxygenase 1 (HO-1) and Total Oxidant-Antioxidant Status in Patients with COVID-19

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ABSTRACT

Introduction: Studies on nuclear factor erythroid 2-related factor 2 (Nrf2) and heme oxygenase-1 (HO-1) levels in COVID-19 patients are limited. This study aimed to investigate the relationship between some biomarkers of oxidant-antioxidant status with COVID-19 disease.

Material and methods: The patients older than 18 years of age who tested positive for SARS CoV-2 PCR (polymerase chain reaction) with clinical symptoms and signs were included in this study. Total antioxidant status (TAS), total antioxidant status (TOS), oxidative stress index (OSI) and HO-1 and Nrf2 levels were analyzed from serum samples taken before and after treatment.

Results: In this study, 16 patients followed up with the diagnosis of COVID-19 were included. 9 (56.3%) of the patients were female and 7 (43.8%) were male. The mean age was 33.75 ± 17.03 years. All patients were symptomatic and were hospitalized to be followed up. It was determined that Nrf2 and HO-1 values increased significantly after treatment. Moreover, there was a significant positive correlation between Nrf2 and TAS values and TAS increases significantly in parallel to an increase in Nrf2, and there was a significant but negative correlation between Nrf2 and TOS and OSI values, and thus an increase in Nrf2 led to a decrease in TOS and OSI values. There was a significant positive correlation between HO-1 and TAS, and TAS increased significantly, as HO-1 increased.

Conclusions: The decrease in TOS and OSI and the increase in Nrf2 and HO-1 during the follow-up period in COVID-19 patients suggest that the body tries to prevent ROS-related oxidative stress via Nrf2 and HO-1 and that oxidative stress may have a key role in the pathophysiology of COVID-19.

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Factor nuclear relacionado con el eritroide 2 (NRF2), hemo oxigenasa 1 (HO-1) y estado oxidante-antoxidante total en pacientes con COVID-19

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RESUMEN

Introducción: Los estudios sobre los niveles del factor 2 relacionado con el factor nuclear eritroide 2 (Nrf2) y la hemo oxigenasa-1 (HO-1) en pacientes con COVID-19 son limitados. Este estudio tuvo como objetivo investigar la relación entre algunos biomarcadores del estado oxidante-antioxidante con la enfermedad COVID-19.

Material y métodos: Se incluyeron en este estudio los pacientes mayores de 18 años que dieron positivo a PCR (reacción en cadena de la polimerasa) de SARS CoV-2 con síntomas y signos clínicos. Se analizaron el estado antioxidante total (TAS), el estado antioxidante total (TOS), el índice de estrés oxidativo (OSI) y los niveles de HO-1 y Nrf2 a partir de muestras de suero tomadas antes y después del tratamiento.

Resultados: En este estudio se incluyeron 16 pacientes seguidos con diagnóstico de COVID-19. 9 (56,3%) de los pacientes eran mujeres y 7 (43,8%) eran hombres. La edad media fue 33,75 ± 17,03 años. Todos los pacientes presentaban síntomas y fueron hospitalizados para seguimiento. Se determinó que los valores de Nrf2 y HO-1 aumentaron significativamente después del tratamiento. Además, hubo una correlación positiva significativa entre los valores de Nrf2 y TAS y TAS aumenta significativamente en paralelo a un aumento en Nrf2, y también hubo una correlación significativa pero negativa entre Nrf2 y los valores de TOS y OSI y, por lo tanto, un aumento en Nrf2 condujo a una disminución en los valores TOS y OSI. Hubo una correlación positiva significativa entre HO-1 y TAS, y TAS aumentó significativamente a medida que aumentaba HO-1.

Conclusiones: La disminución de TOS y OSI y el aumento de Nrf2 y HO-1 durante el período de seguimiento en pacientes con COVID-19 sugieren que el cuerpo intenta prevenir el estrés oxidativo relacionado con ROS a través de Nrf2 y HO-1 y que el estrés oxidativo puede tener un papel clave en la fisiopatología de COVID-19.

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1. INTRODUCTION

World Health Organization (WHO) declared coronavirus disease 2019 (COVID-19) as a global epidemic on March 11, 2020 and more than 220 countries worldwide are affected by the disease and number of cases reached 615 million while 6,524,568 people died due to the disease worldwide (04/10/2022), making this situation a public health crisis [1, 2]. *Coronaviruses* are enveloped Ribonucleic acid (RNA) viruses from the beta-coronavirus genus of the *Coronaviridae* family. SARS coronavirus-2 (SARS-CoV-2) has been defined as the seventh coronavirus that is pathogenic in humans after other types of coronaviruses, which are seasonal HCoV, SARS-CoV, and MERS-CoV [3].

COVID-19 caused by SARS-CoV-2, able to cause different results from an asymptomatic clinical course to acute respiratory distress syndrome (ARDS) or severe pneumonia that may lead to death [4]. COVID-19 is characterized by an abnormal host immune response causing excessive inflammatory responses that are revealed by elevated plasma levels of cytokines, chemokines, and C-reactive protein (CRP). This has fatal consequences, as well as causing multi-organ failure and serious damage to the respiratory system [5].

Reactive oxygen species (ROS) that occurred after physiological and metabolic activities. This ROSs is removed by enzymatic and non-enzymatic anti-oxidative mechanisms to prevent damages that may occur in the organism [6]. Nuclear factor erythroid 2-associated factor 2 (Nrf2) is a transcription factor responsible for the adaptation of cells to oxidative or electrophilic stress. Nrf2 also stimulates expression of heme oxygenase (HO-1) [7]. HO-1 has a crucial role in the antioxidant defense system and iron homeostasis. This enzyme can prevent oxidative deoxyribonucleic acid (DNA) damage, which occurs because of cells being exposed to heat shock, changes in ROS or DNA distribution and cell cycle induced by

pyrrolidine dithiocarbamate [8]. In this study, it was aimed to investigate the relationship of some biomarkers of oxidant-antioxidant status with COVID-19.

2. MATERIAL AND METHODS

2.1. STUDY DESIGN AND POPULATION

The patients older than 18 years of age who tested positive for SARS CoV-2 PCR (polymerase chain reaction) between June 2020 - December 2021 with clinical symptoms and signs in the Infectious Diseases Service of Harran Medical Faculty Hospital were included in this study.

2.2. INCLUSION CRITERIA

Patients who presented to the emergency department with various symptoms and were diagnosed with COVID-19 because of the reverse transcriptase polymerase chain reaction (RT-PCR) test for SARS COV 2 virus positive were included in the study. Within the scope of this study, patients were grouped according to the diagnostic criteria established in the "COVID-19 (2019-nCoV Disease) Guide", which has been published by the Turkish Ministry of Health and is regularly updated with the latest developments. Accordingly, COVID-19 has been classified into four groups. **Group 1** included the presence of mild clinical symptoms; **Group 2** included the presence of respiratory symptoms, fever, and radiological pneumonia findings; **Group 3** included shortness of breath, oxygen saturation ≤ 93% at rest, respiratory rate (RR) ≥ 30/min, and PaO_2/FiO_2 ≤ 300 mmHg; and **Group 4** included clinical shock status, organ failure or respiratory failure requiring mechanical ventilation [9].

2.3. EXCLUSION CRITERIA

Negative results of RT-PCR test for SARS COV 2 virus, diagnosis of COVID-19 who started treatment, history of smoking, hypertension, chronic heart disease, chronic lung disease, malignancy, diabetes mellitus, immunodeficiency or recent trauma were excluded from the study.

2.4. SAMPLING AND ANALYSIS

Nasopharyngeal swab was taken from the patients and RT-PCR test was used.

2.5. STUDY PROTOCOL

During hospitalization and one week after discharge, 2mL of venous blood was taken from the patients and transferred to serum separator tubes (Becton Dickinson, USA). The tubes were centrifuged at 5000 rpm for 5-10 minutes and the obtained serum sample was stored at -80 degrees.

2.6. BLOOD SAMPLING AND ANALYSIS

2.6.1. MEASUREMENT OF NRF2 AND HO-1

Human NFE2L2 ELISA kit (Catalog number: EH3417, BT-LAB) and Human HO1 ELISA kit (Catalog number: EH3234, BT-LAB) were used to analyze Nrf2 and HO-1 levels.

2.6.2. MEASUREMENT OF TOTAL ANTIOXIDANT STATUS (TAS)

Commercial kits (Rel Assay Diagnostic Gaziantep, Turkey) were used in a microplate reader system (Varioskan Lux, Thermo Scientific, USA) to measure total antioxidant status. The data were expressed as mmol Trolox equivalent/L.

2.6.3. MEASUREMENT OF TOTAL OXIDANT STATUS (TOS)

Commercial kits (Rel Assay Diagnostic Gaziantep, Turkey) were used in a microplate reader system (Varioskan Lux, Thermo Scientific, USA) to measure total oxidant status. The data were expressed as μmol H_2O_2 equivalent/L.

2.6.4. CALCULATION OF OXIDATIVE STRESS INDEX (OSI)

Firstly, TAS units results were converted to mmol/L, and then following formula was used: OSI (arbitrary unit) = TOS (mmol H_2O_2 equivalent/L)/TAS (mmol Trolox equivalent/L).

2.6.5. STATISTICAL ANALYSIS

SPSS version 22.0 (SPSS Inc., Chicago, IL) program was used for statistical analysis. Power analysis was performed to determine sample size using G*Power v3.1.9.7. Effect size was selected as 0.75 (to detect the medium-sized differences), α as 0.05 and power of the test as 0.80. Descriptive statistics were summarized as number, median (minimum–maximum), mean, percentage, and standard deviation. Shapiro-Wilk (Since n=16 ≤30) test was used for investigating the conformity of variables to normal distribution. Continuous variables were analyzed with either Paired Samples T-Test or Wilcoxon test depending on homogeneity and distribution of the data. Pearson correlation was used to examine the relationship between parameters. Correlation coefficient (r) was found because of

Pearson correlation analysis. p-value that is less than 0.05 was considered as statistically significant.

2.6.6. ETHICAL CONSIDERATIONS

Approval of the Harran University Faculty of Medicine Ethics Committee was obtained for the study (21/06/2021 HRU/21.12.10).

3. RESULTS

In this study, 16 patients followed up with the diagnosis of COVID-19 in the Infectious Diseases Service were included. 9 (56.3%) of the patients were female and 7 (43.8%) were male. The mean age was 33.75 ± 17.03 (min-max, 18-78, median=27) years. All patients were symptomatic and were hospitalized to be followed up. 14 (87.5%) of them were Group 1 patients while the remaining two (12.5%) were Group 2 patients. There were no patients in Group 3 and Group 4. The most common clinical symptoms were fatigue (n=9, 56.3%), sore throat (n=5, 31.3%), loss of taste and smell (n=5, 31.3%), headache (n=5, 31.3%) and fever (n=4, 25%). The most common pathological laboratory findings were elevated aspartate aminotransferase (AST) (n=9, 56.25%), elevated alanine aminotransferase (ALT) (n=6, 37.5%), elevated CRP (n=6, 37.5%), lymphopenia (n=4, 25%), and elevated lactate dehydrogenase (LDH) (n=3, 18.75%).

While the mean TOS value was 15.99±1.76 μmol H_2O_2 equivalent/L in COVID positive patients in the pre-treatment period, it decreased to 12.75±2.06 μmol H_2O_2 equivalent/L following the treatment (*p<0.05*). Pre-treatment TAS value was 1.26±0.16 mmol Trolox equivalent/L but it increased significantly to 1.52±0.15 mmol Trolox equivalent/L after the treatment **(*p<0.05*)**. Pre-treatment OSI value was 1.28±0.19, it decreased significantly to 0.84±0.18 following the treatment (***p<0.05***). Pre-treatment Nrf2 and HO1 values were 0.22±0.04 and 0.32±0.10 μg/mL, respectively, they increased significantly to 0.38±0.05 and 0.47±0.14 μg/mL after treatment (*p<0.05*) (Table 1).

Table 1: Assessment of COVID-19 patients based on their treatment status

COVID-19 (+)	Pre-treatment (n:16)	Post-treatment (n:16)	[a]*p*
TAS mmol Trolox equivalent/L	1.26±0.16	1.52±0.15	*0.00*
TOS μmol H_2O_2 equivalent/L.	15.99±1.76	12.75±2.06	*0.00*
OSI OSI (arbitrary unit)=TOS /TAS	1.28±0.19	0.84±0.18	*0.00*
Nrf2 (μg/mL)	0.22±0.04	0.38±0.05	*0.00*
HO-1 (μg/mL)	0.32±0.10	0.47±0.14	*0.00*

Values are expressed as mean±standard deviation.
[a]p: [a]Paired Samples t-Test; TAS: Total antioxidant status; TOS: Total oxidant status; OSI: Oxidative stress index; Nrf2: Nuclear factor erythroid 2-related factor 2; HO-1: Heme oxygenase-1.

The correlation of Nrf2 and HO-1 levels with TAS, TOS and OSI were examined. A statistically significant (***p<0.05***) positive correlation was determined between Nrf2 and TAS values, and TAS increases significantly in parallel to an increase in Nrf2. On the other hand, a statistically significant (*p<0.05*) but negative correlation determined between Nrf2 and OSI and TOS values, and thus an increase in Nrf2 led to a decrease in OSI and TOS values. A statistically significant (*p<0.05*) positive correlation determined between HO-1 and TAS, and it was determined that as HO-1 increased, TAS increased significantly. Also, a statistically significant

Table 2: Correlation of Nrf2 and HO-1 levels with TAS, TOS and OSI in COVID-19 patients

		Nrf2	HO-1
TAS		.690	.419
		.000	.017
		32	32
TOS		-.524	-.355
		.002	.046
		32	32
OSI		-.701	-.465
		.000	.007
		32	32

TAS: Total antioxidant status; TOS: Total oxidant status; OSI: Oxidative stress index; Nrf2: Nuclear factor erythroid 2-related factor 2; HO-1: Heme oxygenase-1.

($p<0.05$) but negative correlation between HO-1 and TOS and OSI values (Table 2).

4. DISCUSSION

In this study, we examined the relationship between COVID-19 and oxidative stress. TOS and OSI levels decreased after the treatment while TAS, Nrf2, and HO increased. These results were statistically significant. The decrease in oxidative stress indicators throughout the process in COVID-19 and the increase in TAS, which is an indicator of anti-oxidative state, as well as in Nrf2 and HO-1, which are indicators of anti-oxidative defense response, suggest that the body tries to prevent ROS-related oxidative stress via Nrf2 and HO-1. Therefore, it could be suggested that oxidative stress may have a key role in COVID-19.

Oxidative stress is defined as "the situation in which oxidation occurs due to the loss of balance between the body's antioxidant systems" [10]. ROS and reactive nitrogen species (RNS) are by products of several cellular processes. Under normal physiological conditions, the balance between ROS and antioxidant levels is maintained at the cellular level. However, these powerful oxidants (free radicals) may pose harmful effects when the redox balance is lost; they are often associated with the onset of various lifestyle diseases. Such harmful effects are caused by free radicals that attack various cells and contain uneven oxygen and nitrogen damaging DNA, proteins, and lipids [11].

Although various measurement methods have been developed for TOS and TAS, there is no generally accepted reference method yet. In this study, we used a novel automatic measurement method developed by Erel to determine total antioxidant status and total oxidant status of the plasma. This method is advantageous since it is simple, inexpensive, reliable, and very sensitive. Considering the indices, the literature provides some but the most widely used one is the OSI, which was proposed in 2003. This index has changed little over time, but all conducted studies conclude and agree that it is a ratio between TOS and TAS expressed as arbitrary units [12-16]. Karkhanei et al. [17] performed a case-control study aiming to investigate the association between some oxidative stress biomarkers and COVID-19, and the patients were divided into four groups as non-intensive care, intensive care and intubated, intensive care but non-intubated, and healthy without COVID-19 diagnosis. This study found TOS to be significantly higher in those three groups compared to the control group. On the other hand, TAS level was not different in non-intensive care patients compared to the control group but was significantly higher in patients followed up in intensive care unit compared to the control group. Mehri et al. [18] found that the mean serum TOS concentration was significantly higher in COVID-19 patients when compared to the control group and they stated that this elevation might have an important role in the mechanism of disease development. Aykaç et al. [19] determined that serum TOS levels were significantly higher in patients diagnosed with COVID-19. In a case-control study conducted by Gümüş et al. [20] to investigate the relationship between COVID-19 and oxidative stress in pediatric patients, Nrf2 and TAS levels were found to be lower and TOS and OSI levels higher in the patient group, and it was stated that this might be related to tissue damage in COVID-19. In our study, we determined that TOS value decreased after the treatment compared to pre-treatment. The pre-treatment TAS value increased significantly in the post-treatment period. The pre-treatment OSI value decreased significantly. Accordingly, the decrease in TOS, which is among oxidative stress parameters, and the increase in TAS, which reflects the anti-oxidative state, suggest that anti-oxidative mechanisms step in during the disease and endeavor to reduce oxidative damage.

Various studies have been conducted on the relationship between HO-1 enzyme level and viral infections. Su et al. [21] carried out a study comparing the relationship between desaturation and heme oxygenase in patients with COVID-19 diagnosis. The authors observed that heme and HO-1 enzyme levels increased significantly in the low SpO_2 group, and they believed that the increased HO-1 in the low oxygenation group may reduce inflammation and provide benefit in terms of survival in this group of patients. L.-L. Ma et al. [22] emphasized the antiviral effect of HO-1 against influenza viruses. The authors of the relevant study demonstrated that cobalt protoporphyrin (CoPP), a potent inducer of HO-1 like hemin, interacts with IRF3 and HO-1 and then inhibits the replication of influenza A virus by expression of IFNa/β. In another study, the same mechanism was observed in the reduction of viral replication and lung inflammation following HO-1 induction and IFNa/β expression in the infected lung in human respiratory syncytial virus infection [23]. HO-1-mediated type I IFN response is assumed to be able to control many other viral infections such as hepatitis B/C virus, Ebola virus, and human immunodeficiency virus by inhibiting virus replication [24]. In our study, we detected that the pre-treatment HO-1 increased significantly in the post-treatment period. This result suggests that HO-1 enzyme may have antiviral effects in the pathogenesis of COVID-19 and may contribute to the reduction of oxidative stress.

As a transcription factor, Nrf2 regulates certain genes

directly defending against oxidative stress, including catalases, numerous peroxidases, superoxide dismutase, and enzymes synthesizing glutathione [25]. Attending to the human coronavirus HCoV-229E associated with cold and lung disease, deficient expression of Nrf2 target gene glucose-6-phosphate dehydrogenase (G6PDH) increases ROS production, viral gene expression, and particle production. It is also observed that lung biopsies of COVID-19 patients showed that the Nrf2 pathway is suppressed and the pharmacological inducers of Nrf2 inhibit the replication of SARS-CoV2 and the inflammatory response [26-29]. The current study demonstrated significantly increased Nrf2 after treatment of COVID-19. This increase in Nrf2 level points out that the Nrf2 pathway may be an important factor in reducing oxidative stress in COVID-19 infection. It is also known that Nrf2 has a role in stimulating the expression of HO-1 [7].

There are many studies investigating the inflammatory parameters in COVID-19 prognosis [30-32]. However, this study is one of the rare studies investigating oxidative stress in COVID-19. Similarly, Binici et al. [31] investigated superoxide dismutase, glutathione peroxidase, glutathione, total thiol, natural thiol, disulfide, oxidative DNA damage, and malondialdehyde levels in 35 COVID-19 cases and 35 control volunteers. In this study, serum superoxide dismutase, glutathione peroxidase, malondialdehyde, 8-hydroxy-2-deoxyguanosine/10^6, disulfide levels were higher, and glutathione, total thiol, and natural thiol levels were lower in COVID-19 group compared to the healthy control group. In addition, there was a negative correlation between 8-hydroxy-2-deoxyguanosine/10^6 deoxyguanosine and glutathione, natural thiol, and total thiol, and a positive correlation with disulfide [30]. Our results showed that the statistically significant increase in both Nrf2 and HO-1 levels supports our anticipation.

The important limitations of this study were the small patient population, the absence of severe patients, and the inability to compare with healthy volunteers.

5. CONCLUSIONS

COVID-19 is still a global problem due to the resulting high morbidity and mortality. Considering the pre-treatment and post-treatment results relating to TAS, TOS, OSI, Nrf2, and HO-1 oxidative stress biomarkers in our study, we think that oxidative stress may play an important role in the pathophysiology of COVID-19. However, there were no clinically severe patients in our study. Large-scale studies including patients with severe COVID-19 are needed.

6. CONFLICT OF INTERESTS

The authors have no conflict of interest to declare. The authors declared that this study has received no financial support.

7. REFERENCES

1. Msemburi W, Karlinsky A, Knutson V, Aleshin-Guendel S, Chatterji S, Wakefield J. The WHO estimates of excess mortality associated with the COVID-19 pandemic. Nature. 2023;613(7942):130-7. doi: 10.1038/s41586-022-05522-2.

2. COVID-19 Excess Mortality Collaborators. Estimating excess mortality due to the COVID-19 pandemic: a systematic analysis of COVID-19-related mortality, 2020-21. Lancet. 2022;399(10334):1513-36. doi: 10.1016/S0140-6736(21)02796-3.

3. Rabi FA, Al Zoubi MS, Kasasbeh GA, Salameh DM, Al-Nasser AD. SARS-CoV-2 and Coronavirus Disease 2019: What We Know So Far. Pathogens. 2020;9(3):231. doi: 10.3390/pathogens9030231.

4. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020 Feb 15;395(10223):497-506. doi: 10.1016/S0140-6736(20)30183-5.

5. Pincemail J, Cavalier E, Charlier C, Cheramy-Bien JP, Brevers E, Courtois A, et al. Oxidative Stress Status in COVID-19 Patients Hospitalized in Intensive Care Unit for Severe Pneumonia. A Pilot Study. Antioxidants (Basel). 2021;10(2):257. doi: 10.3390/antiox10020257.

6. Robert L, Labat-Robert J. Stress in biology and medicine, role in aging. Pathol Biol (Paris). 2015;63(4-5):230-4. doi: 10.1016/j.patbio.2015.07.008.

7. Zinovkin RA, Grebenchikov OA. Transcription Factor Nrf2 as a Potential Therapeutic Target for Prevention of Cytokine Storm in COVID-19 Patients. Biochemistry (Mosc). 2020;85(7):833-7. doi: 10.1134/S0006297920070111.

8. Alcaraz MJ, Fernández P, Guillén MI. Anti-inflammatory actions of the heme oxygenase-1 pathway. Curr Pharm Des. 2003;9(30):2541-51. doi: 10.2174/1381612033453749.

9. General Directorate of Public Health Republic of Turkey Ministry of Health [Internet]. COVID-19 (SARS-CoV-2 Infection) Guide. Scientific Committee Study. Available from: https://covid19bilgi.saglik.gov.tr/depo/rehberler/COVID-19_Rehberi.pdf (accessed 10 May 2022).

10. Sies H. Oxidative stress: a concept in redox biology and medicine. Redox Biol. 2015;4:180-3. doi: 10.1016/j.redox.2015.01.002.

11. Suhail S, Zajac J, Fossum C, Lowater H, McCracken C, Severson N, et al. Role of Oxidative Stress on SARS-CoV (SARS) and SARS-CoV-2 (COVID-19) Infection: A Review. Protein J. 2020;39(6):644-56. doi: 10.1007/s10930-020-09935-8.

12. Tarpey MM, Wink DA, Grisham MB. Methods for detection of reactive metabolites of oxygen and nitrogen: in vitro and in vivo considerations. Am J Physiol Regul Integr Comp Physiol. 2004;286(3):R431-44. doi: 10.1152/ajpregu.00361.2003.

13. Erel O. A new automated colorimetric method for measuring total oxidant status. Clin Biochem. 2005;38(12):1103-11. doi: 10.1016/j.clinbiochem.2005.08.008.

14. Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin Biochem. 2004;37(4):277-85. doi: 10.1016/j.clinbiochem.2003.11.015.

15. Harma M, Harma M, Erel O. Increased oxidative stress in patients with hydatidiform mole. Swiss Med Wkly. 2003;133(41-42):563-6. doi: 10.4414/smw.2003.10397.

16. Sánchez-Rodríguez MA, Mendoza-Núñez VM. Oxidative Stress Indexes for Diagnosis of Health or Disease in Humans. Oxid Med Cell Longev. 2019;2019:4128152. doi: 10.1155/2019/4128152.

17. Karkhanei B, Talebi Ghane E, Mehri F. Evaluation of oxidative stress level: total antioxidant capacity, total oxidant status and glutathione activity in

patients with COVID-19. New Microbes New Infect. 2021;42:100897. doi: 10.1016/j.nmni.2021.100897.

18. Mehri F, Rahbar AH, Ghane ET, Souri B, Esfahani M. Changes in oxidative markers in COVID-19 patients. Arch Med Res. 2021;52(8):843-9. doi: 10.1016/j.arcmed.2021.06.004.

19. Aykac K, Ozsurekci Y, Yayla BCC, Gurlevik SL, Oygar PD, Bolu NB, et al. Oxidant and antioxidant balance in patients with COVID-19. Pediatr Pulmonol. 2021;56(9):2803-10. doi: 10.1002/ppul.25549.

20. Gümüş H, Erat T, Öztürk İ, Demir A, Koyuncu I. Oxidative stress and decreased Nrf2 level in pediatric patients with COVID-19. J Med Virol. 2022;94(5):2259-64. doi: 10.1002/jmv.27640.

21. Su WL, Lin CP, Hang HC, Wu PS, Cheng CF, Chao YC. Desaturation and heme elevation during COVID-19 infection: A potential prognostic factor of heme oxygenase-1. J Microbiol Immunol Infect. 2021;54(1):113-6. doi: 10.1016/j.jmii.2020.10.001.

22. Ma LL, Zhang P, Wang HQ, Li YF, Hu J, Jiang JD, et al. heme oxygenase-1 agonist CoPP suppresses influenza virus replication through IRF3-mediated generation of IFN-α/β. Virology. 2019;528:80-8. doi: 10.1016/j.virol.2018.11.016.

23. Espinoza JA, León MA, Céspedes PF, Gómez RS, Canedo-Marroquín G, Riquelme SA, et al. Heme Oxygenase-1 Modulates Human Respiratory Syncytial Virus Replication and Lung Pathogenesis during Infection. J Immunol. 2017;199(1):212-23. doi: 10.4049/jimmunol.1601414.

24. Rossi M, Piagnerelli M, Van Meerhaeghe A, Zouaoui Boudjeltia K. Heme oxygenase-1 (HO-1) cytoprotective pathway: A potential treatment strategy against coronavirus disease 2019 (COVID-19)-induced cytokine storm syndrome. Med Hypotheses. 2020;144:110242. doi: 10.1016/j.mehy.2020.110242.

25. McCord JM, Hybertson BM, Cota-Gomez A, Gao B. Nrf2 activator PB125® as a carnosic acid-based therapeutic agent against respiratory viral diseases, including COVID-19. Free Radic Biol Med. 2021;175:56-64. doi: 10.1016/j.freeradbiomed.2021.05.033.

26. Cuadrado A, Pajares M, Benito C, Jiménez-Villegas J, Escoll M, Fernández-Ginés R, et al. Can Activation of NRF2 Be a Strategy against COVID-19? Trends Pharmacol Sci. 2020;41(9):598-610. doi: 10.1016/j.tips.2020.07.003.

27. Wu YH, Tseng CP, Cheng ML, Ho HY, Shih SR, Chiu DT. Glucose-6-phosphate dehydrogenase deficiency enhances human coronavirus 229E infection. J Infect Dis. 2008;197(6):812-6. doi: 10.1086/528377.

28. Olagnier D, Farahani E, Thyrsted J, Blay-Cadanet J, Herengt A, Idorn M, et al. SARS-CoV2-mediated suppression of NRF2-signaling reveals potent antiviral and anti-inflammatory activity of 4-octyl-itaconate and dimethyl fumarate. Nat Commun. 2020;11(1):4938. doi: 10.1038/s41467-020-18764-3.

29. Gorse GJ, O'Connor TZ, Hall SL, Vitale JN, Nichol KL. Human coronavirus and acute respiratory illness in older adults with chronic obstructive pulmonary disease. J Infect Dis. 2009;199(6):847-57. doi: 10.1086/597122.

30. Turgunova L, Mekhantseva I, Bacheva I, Amirkhanova D, Butyugina M, Samoilova N. Prognostic factors for the severe course of COVID-19 in the different COVID-19 peak periods in Central Kazakhstan. J Clin Med Kaz. 2022;19(4):53-8. doi: 10.23950/jcmk/12293.

31. Binici İ, Alp HH, Huyut Z, Gürbüz E, Günbatar H, Akmeşe Ş, et al. The Status of Antioxidants and Oxidative Damage in Patients with COVID-19. Ahi Evran Medical Journal. 2023;7(1):114-23. doi: 10.46332/aemj.1152479.

32. Küçük U, Alkan Çeviker S, Şener A. Relationship between in-hospital mortality and inflammation markers in COVID-19 patients with the diagnosis of coronary artery disease. J Contemp Med. 2021;11(3):267-71. doi: 10.16899/jcm.869095.