JOURNAL OF CLINICAL MEDICINE OF KAZAKHSTAN (E-ISSN 2313-1519)
Original Article DOI: https://doi.org/10.23950/jcmk/13142

Role of pyroptosis in COVID-19

Mehmet Çelik[1], Mehmet Reşat Ceylan[1], Mahmut Alp Karahan[2], İsmail Koyuncu[3], Nevin Güler Dinçer[4], Sevil Alkan[5]

[1]Department of Infectious Diseases and Clinical Microbiology, Faculty of Medicine, University of Harran, Şanlıurfa, Turkey
[2]Department of Anesthesiology, ŞAnlıurfa Mehmet Akif İNAN Training and Research Hospital, ŞAnlıurfa, Turkey
[3]Department of Medical Biochemistry, Faculty of Medicine, University of Harran, Şanlıurfa, Turkey
[4]Department of Statistics, Faculty of Science, University of Muğla Sıtkı Koçman, Muğla, Turkey
[5]Department of Infectious Diseases, Faculty of Medicine, Canakkale Onsekiz Mart University, Canakkale, Turkey

Received: 2023-01-27.
Accepted: 2023-03-07

J Clin Med Kaz 2023; 20(2):39-45

Corresponding author:
Mehmet Çelik.
E-mail: dr.mcelik12@gmail.com;
ORCID: 0000-0002-0583-929X

Abstract

Objectives: In this study, it was investigated the relationship between of gasdermin-D, caspase-1, IL-1β and NLRP3, which are biomarkers that play an important role in the pyroptosis and COVID-19.

Material and methods: This study was carried out with 58 participants, 28 (48.28%) of whom were diagnosis of COVID-19, and 30 (51.72%) of whom were healthy volunteers (control group).

Results: There were no statistically significant differences between the gasdermin-D, caspase-1, IL-1β and NLRP3 levels as a result of all statistical comparisons performed. However, IL-1β values both at the discharge period and at hospitalization period were considerably higher than those of control group. At discharge period, IL-1β values of the patients with severe COVID-19 category had higher than moderate patients, and the patients with moderate than the patients with the mild patients.

Conclusion: It was observed that IL-1β, which is one of the cytokines released as a result of cell death in the pyroptosis mechanism, was higher in the COVID-19 patients both the hospitalization and discharge periods compared to the control group. Although not statistically significant these results could support the relationship between the pyroptosis and COVID-19.

Key words: COVID-19, interleukin-1β, pyroptosis

Introduction

Coronaviruses are enveloped RNA viruses belonging to the beta-coronavirus genus in the *Coronaviridae* family [1]. After the pneumonia clustering detected on 31 December 2019 was determined to be caused by a new Coronavirus never before seen in humans, this Coronavirus was defined as SARS-CoV-2 [2]. SARS-CoV-2 has been described as the 7th Coronavirus, that is pathogenic in humans [1]. Coronavirus disease 2019 (COVID-19) was declared a pandemic by the World Health Organization (WHO) on March 11, 2020 [3]. The disease has spread to more than 220 countries the worldwide. The number of cases reached 515 million and deaths reached 6,254,140 worldwide [4].

While the disease may progress asymptomatically, it can also be seen in respiratory failure requiring mechanical ventilation, sepsis, septic shock and multiple organ failure [5]. COVID-19 is characterized by an abnormal host immune response, manifested by high blood cytokine, chemokine, and C-reactive protein (CRP) levels [6]. And it has been shown in many previous studies that COVID-19 can have multi-system involvement (cardiac, gastrointestinal, etc.) [7-14].

In addition to known cell death mechanisms such as necrosis, autophagy and apoptosis, a new type of cell death, the pyroptosis, has been defined in recent years. Pyroptosis is a type of programmed and inflammatory necrosis that occurs due to caspase (cysteinyl aspartate specific proteinase) activation and pore formation in the cell membrane conducted by the gasdermin protein

family. Pyroptosis results lytic cell death accompanied by the release of various inflammatory factors by inducing amplification of the cascade and of the inflammatory response. In addition, it is an important immune defense mechanism in the body that resists the invasion of external pathogens and plays a role in perceiving internal pathogenic signals in cells [15]. Various evidences have been shown to support that in severe COVID-19 cases, there are inflammasome activation, the pyroptosis and their critical roles. It was found that the pyroptosis is associated with caspase-1 activation, gasdermin-D (GSDMD) cleavage and increased levels of proinflammatory cytokines in primary monocytes and macrophages of COVID-19 patients [16-18]. In this study, it was investigated the relationship between of GSDMD, caspase-1, Interleukin-1β (IL-1β) and NOD-like receptor family pyrin domain-containing 3 (NLRP3), which are biomarkers that play an important role in the pyroptosis and COVID-19.

Material and methods

Study population and participant groups

The patients over 18 years of age, who were diagnosed with COVID-19 as a result of clinical signs, symptoms and laboratory tests and who were hospitalized at the COVID-19 ward of Harran University Medical Faculty Hospital and the control group consisting of healthy volunteers without any symptoms or underlying disease included in the study. The patient group consisted of the participants were diagnosed with a positive real-time reverse transcription-polymerase chain reaction (rRT-PCR) in addition to various symptoms and findings and were followed up in the hospital. The patients were grouped grounded on the "COVID 19 (2019-nCoV Disease) Guidelines" published by the Turkish Ministry of Health. According to this guideline, patients diagnosed with COVID-19 were divided into four subgroups; Group 1 was classified as mildly symptomatic, Group 2 as symptomatic (radiological involvement in addition to fever and respiratory symptoms), Group 3 as symptomatic (dyspnea, oxygen saturation ≤93% at rest) and Group 4 as critically ill (respiratory failure, clinical shock or organ failure requiring mechanical ventilation) [19].

Biochemical analyses

Blood samples were collected from both COVID-19 patients and healthy volunteers. Serum samples were obtained by centrifugation of the blood samples taken at 3500 rpm for 10 minutes and these serum samples were stored in the refrigerator at -80˚C until the day of the study. GSDMD (Gasdermin D Elisa Kit (BT Lab Catalog no: E6838Hu)), caspase-1 (BT Lab; E2248Hu), IL-1β (Interleukin 1 Beta; BT Lab E0143Hu) and NLRP3 (Human Nlr Family Pyrin Domain, BT Lab E3886Hu)) levels were performed according to the commercially purchased ELISA kit protocol. After adding 100 µl of a serum sample to the 96 plates in the kit, it was incubated at 37° C for 90 minutes. After incubation, the plate was emptied, washed twice with a washing solution, and dried. 100 µl Biotin-labeled antibody was added and incubated at 37°C for one hour. After incubation, the plate was drained and washed three times with a washing solution and dried. 100 µl HRP-Streptavidin Conjugate was added and incubated at 37°C for 30 minutes. After incubation, the plate was drained and washed five times with wash solution and dried. Then 90 µl TMB Substrate was added and incubated at 37°C in the dark. 50µl of Stop Solution was added after color formation was observed. Data were obtained by reading the plates in a microplate reader (Biotek-Cytation-1) at 450 nm absorbance.

Statistical analyses

SPSS version 22.0 was employed in the statistical analysis (SPSS Inc., Chicago, IL). G*Power v3.1.9.4 was implemented to conduct a power analysis in order to determine the sample size. There were four ways to sum up descriptive statistics: number, percentage, mean, and standard deviation (S.D). Continuous variables were examined to see if they adhered to the normal distribution using the Kolmogorov-Smirnov test. With the help of the paired samples t-test and the two independent sample t-test, continuous variables with the normal distribution were examined. The variables that did not exhibit a normal distribution were subjected to the Mann-Whitney U test and the Wilcoxon sign test. For comparing more than two continuous variables, use the Kruskal-Wallis test. For all statistical tests, p 0.05 was accepted as the significance level.

Results

A total of 58 participants, including 28 (48.28%) COVID-19 patients hospitalized in the COVID-19 ward and 30 (51.72%) healthy controls, were included in the current study.

15 (53.6%) of the patients in the COVID-19 group were female and 13 (46.4%) were male. 15 (50%) of the patients in the control group were female and 15 (50%) male. 7 (25%) of the patients were categorized in Group 1, 14 (50%) in Group 2 and 7 (25%) in Group 3. There were no patients in Group 4.

The most common complaints of COVID-19-infected patients at the time of admission were weakness-fatigue (n=25, 89.3%), cough (n=23, 82.1%), muscle-joint pain (n=20, 71.4%), shortness of breath (n=19, 67.9%) and fever (n=13, 46.4%). Comorbidities were present in 15 (53.6%) of COVID-19-infected patients. The most common comorbidities were diabetes mellitus (n=7, 25%) and hypertension (n=6, 21.4%). Comparison of the laboratory parameters of the patients during hospitalization and discharge periods showed statistically significant differences in platelet, lactate dehydrogenase (LDH), CRP, alanine aminotransferase (ALT), urea, and creatinine levels (Table 1).

However, we found no statistically significant difference between GSDMD, caspase-1, IL-1β, and NLRP3 values during hospitalization and discharge periods. In addition, there was no statistically significant difference between the biomarkers of hospitalized COVID-19 patients and the biomarkers of the control group. However, it was observed that all of four biomarkers, more prominently at IL-1β level, were found to be higher in the patient group than the control group (Table 2).

The Kruskal-Wallis was performed to detect whether difference of GSDMD, caspase-1, IL-1β and NLRP3 levels were statistically significant between the COVID-19 patient groups. As a result of the test, it was determined that there were no statistically differences between the biomarkers according to the patient's groups. Although it could not be determined the significant differences, there were remarkable differences between the means of IL-1β values of the patient's groups. It was concluded that the reason of not determining the statistically significant differences was that the standard deviations were high (Table 3).

In the COVID-19 patient group, no statistically significant difference was found between the GSDMD, IL-1β, caspase-1, and NLRP3 values of patients with lymphopenia, leukopenia, and neutropenia at hospitalization and discharge. However, in patients with lymphopenia and leukopenia, while IL-1β and GSDMD levels increased at the discharge period, it was observed that caspase-1 and NLRP3 levels decreased (Table 4).

Table 1 Laboratory parameters of COVID-19 infected inpatients at admission day and discharge day

Parameters	Admission day (n=28)		Discharge day (n=28)		p
	Mean	S.D	Mean	S.D	
Leucocyte (cells/ mm3)	5835.67	2390.26	7060.42	3333.037	0.064
Neutrophil (cells/ mm3)	4206.67	2247.06	5283.75	3027.29	0.088
Lymphocyte (cells/ mm3)	1139.67	552.98	1516.79	950.75	0.079
Platelet (cells/mm3)	204.42	74.13	300.46	160.03	0.001
HGB (g/dL)	13.80	1.68	13.44	1.95	0.132
PT (seconds)	11.79	1.87	11.77	2.18	0.755
INR	0.95	0.15	0.96	0.18	0.776
D-Dimer (µg/mL)	0.88	0.71	1.20	2.89	0.255
Fibrinogen(mg/dL)	422	251.72	409.57	163.43	0.913
Ferritin (ng/mL)	391.13	530.29	657.00	611.75	0.262
LDH (U/L)	355.36	169.41	236.73	48.99	0.005
CRP (mg/dL)	5.47	7.34	1.84	2.18	0.011
ALT (U/L)	39.08	29.90	111.75	92.100	0.000
AST (U/L)	39.88	13.34	83.00	53.53	0.062
Urea (mg/dL)	31.58	12.17	36.88	10.99	0.031
Creatine (mg/dL)	0.86	0.23	0.75	0.16	0.006
Albumin (g/dL)	4.16	0.63	4.29	2.26	0.790

HGB: Hemoglobin, AST: Aspartate aminotransferase, PT: Prothrombin time, INR: International Normalized Ratio, SD: standard deviation

Table 2 Comparison of GSDMD, Caspase-1, IL-1β and NLRP3 values of case and control groups on hospitalization day and discharge day

	Admission day (n=58)		Discharge day (n=58)		p
	Mean	S.D	Mean	S.D	
GSDMD	4.24	1.33	4.16	1.43	0.85
Caspase-1	5.24	3.86	5.92	5.71	0.96
IL-1β	928.77	878.85	1335.45	1638.85	0.73
NLRP3	99.66	50.17	97.41	49.90	0.34
Comparison of biomarkers of the COVID-19 group with the control group on admission day					
	Control (n=30)		COVID-19 patients' values on admission day (n=28)		p
	Mean	S.D	Mean	S.D	
GSDMD	3.67	0.98	4.24	1.33	0.30
Caspase-1	5.23	3.73	5.24	3.86	0.53
IL-1β	828.06	613.02	928.77	878.85	0.94
NLRP3	86.57	34.48	99.66	50.17	0.79
Comparison of biomarkers of the COVID-19 group with the control group on disharge day					
	Control (n=30)		COVID-19 patients' values on a disharge day (n=28)		p
	Mean	S.D	Mean	S.D	
GSDMD	3.67	0.98	4.16	1.43	0.13
Caspase-1	5.23	3.73	5.92	5.71	0.73
IL-1β	828.06	613.02	1335.45	1638.77	0.98
NLRP3	86.57	34.48	97.41	49.90	0.97

It was compared biomarkers of patients with LDH>250 U/L and those with LDH<=250 U/L at the hospitalization period. As a result of comparisons, it was found that while IL-1β and caspase-1 values were higher at the discharge period, GSDMD and NLRP3 values were lower. However, these differences were not found statistically significant. When the biomarkers of patients with and without the comorbidity were compared at the hospitalization and discharge periods, it was observed that there were no statistically significant differences between GSDMD, caspase-1, IL-1β and NLRP3 values. GSDMD, caspase-1, IL-1β, and NLRP3 values were higher in patients with comorbidities, both at the hospitalization and the discharge (Table 5).

Table 3 GSDMD, Caspase-1, IL-1β, NLRP3 values on the day of admission and discharge days

	GSDMD, Caspase-1, IL-1β, NLRP3 values on the day of admission						
	Group 1 (mildly symptomatic) (n=7)		Group 2 (symptomatic) (radiological involvement in addition to fever and respiratory symptoms) (n=14)		Group 3 (symptomatic (dyspnea, oxygen saturation ≤93% at rest)) (n=7)		p
	Mean	S.D	Mean	S.S	Mean	S.D	
GSDMD	3.61	0.92	4.37	1.08	4.60	1.98	0.53
Caspase-1	4.05	2.16	5.65	3.75	5.61	5.47	0.57
IL-1β	527.31	216.21	1141.90	956	903.96	1073.83	0.10
NLRP3	85.31	42.80	107.37	46.16	98.59	67.05	0.39
	GSDMD, Caspase-1, IL-1β, NLRP3 values on the day of discharge						
	Group 1 (mildly symptomatic) (n=7)		Group 2 (symptomatic) (radiological involvement in addition to fever and respiratory symptoms) (n=14)		Group 3 (symptomatic (dyspnea, oxygen saturation ≤93% at rest)) (n=7)		p
	Mean	S.D	Mean	S.D	Mean	S.D	
GSDMD	4.59	0.88	3.75	1.36	4.55	1.89	0.15
Caspase-1	3.77	1.59	4.95	3.62	10.03	9.40	0.42
IL-1β	594.44	286.55	1185.41	1232.72	2376.52	2598.63	0.61
NLRP3	83.92	36.79	97.44	50.06	110.84	63.22	0.69

Table 4 GSDMD, Caspase-1, IL-1β, NLRP3 values on the day of admission and discharge days in COVID-19 patients with lymphopenia,leukopenia and neutropenia

GSDMD, Caspase-1, IL-1β, NLRP3 values on the day of admission and discharge days in COVID-19 patients with lymphopenia (n =28)					
	Admission day		Discharge day		p
	Mean	S.D	Mean	S.D	
GSDMD	4.17	1.51	4.67	1.54	0.64
Caspase-1	6.00	4.66	5.94	5.12	0.51
IL-1β	970.05	854.26	1772.89	2118.20	0.12
NLRP3	109.97	58.79	104.36	59.17	0.35
GSDMD, Caspase-1, IL-1β, NLRP3 values on the day of admission and discharge days in COVID-19 patients with leukopenia (n=14)					
	Admission day		Discharge day		p
	Mean	S.D	Mean	S.D	
GSDMD	3.91	1.26	4.46	1.23	0.48
Caspase-1	4.37	3.86	4.30	3.78	0.89
IL-1β	876.04	982.73	978.09	1317.66	0.58
NLRP3	87.73	41.70	79.32	35.59	0.16
GSDMD, Caspase-1, IL-1β, NLRP3 values on the day of admission and discharge days in COVID-19 patients with neutropenia (n=7)					
	Admission day		Discharge day		p
	Mean	S.D	Mean	S.D	
GSDMD	3.30	0.28	5.60	0.62	0.11
Caspase-1	2.49	0.88	2.49	0.33	1.00
IL-1β	489.05	292.10	390.02	48.74	1.00
NLRP3	69.31	22.76	63.26	10.17	1.00

Table 5 Comparison of admission and discharge day biomarkers of patients with and without comorbidity

Comparison of the admission day biomarkers of patients with and without comorbidity					
	With Comorbidity (n=15)		Without Comorbidity(n=13)		p
	Mean	S.D	Mean	S.D	
GSDMD	4.27	1.49	4.21	1.18	0.91
Caspaz-1	5.30	4.29	5.17	3.48	0.93
IL-1β	1099.22	1124.30	732.09	430.00	0.79
NLRP3	88.61	41.92	112.41	57.30	0.22
Comparison of the discharge biomarkers of patients with and without comorbidity					
	With Comorbidity (n=15)		Without Comorbidity (n=13)		p
	Mean	S.D	Mean	S.D	
GSDMD	4.44	1.68	3.83	1.03	0.25
Caspase-1	6.83	6.72	4.88	4.29	0.86
IL-1β	1421.72	1532.37	1235.90	1811.90	1.00
NLRP3	89.72	41.95	106.28	58.22	0.44

Discussion

The process of apoptosis was the first type of programmed cell death to be identified, and in most circumstances, caspase-3 and -7 ensure that it is immune-silent. In contrast, the lytic cell death mechanisms of necroptosis and pyroptosis permit the release of potential immunostimulatory chemicals. According to genetic evidence, these cell death pathways can initiate powerful inflammatory responses in vivo, which may contribute to the pathology of numerous inflammatory diseases. In some cases, the bystander DAMPs released after pyroptosis and necroptosis may be less inflammatory than if the cell did not get the cues for suicide. This may reflect cell-type and stimulus-specific pyroptotic and necroptotic signaling scenarios [20-24].

In this study, GSDMD, caspase-1, IL-1β and NLRP3 biomarkers were studied in patients and healthy volunteers with aim of examining the relationship between pyroptosis and COVID-19. In our study, it was detected that there were no statistically significant differences between the COVID-19 and control groups. When the reason of not detecting the significant differences was investigated, it was determined that the deviations between the biomarker values at the hospitalization and the discharge periods were high (high standard deviation) and it is concluded that these results could be caused by the limited population size. Although there were no statistically significant differences, the distribution of some biomarkers, especially IL-1β, between the groups was considered remarkable. Some findings can be summarized as follows: IL-1β level was found to be higher both at the hospitalization and at the discharge periods of COVID-19 patients when compared with the control group. This is thought to be an important indicator of the interaction between COVID-19 and the pyroptosis mechanism. When the patients with high CRP and those without CRP were compared at the hospitalization period, it was observed that all four parameters were higher in those with high CRP values. This suggests that there is a parallelism between the level of inflammation and pyroptosis. At the hospitalization period, the patients with high LDH levels had higher levels of IL-1β. It was observed that IL-1β levels increased as the severity of the COVID-19 increased according to observations obtained at the discharge period. Besides, all four biomarkers were higher in patients with comorbidity both at the hospitalization and at the discharge periods compared to those without comorbidity. This result suggested that other underlying diseases or factors may affect pyroptosis.

Induction of NOD-like receptor (nucleotide-binding oligomerization domain) activation by pathogen or alarmin results in activation of caspase-1. The proteolytic activation of caspase-1 catalyzes the maturation and secretion of proinflammatory cytokines, notably IL-1β and IL-18. NLRP3, the best described among inflammasomes, has been associated with many diseases from autoinflammatory diseases to neurological disorders. NLRP3 also plays a role in antiviral responses and viral diseases [25,26]. There are six members of the gasdermin protein family, and each of them has different functions in human tissues and organs [27,28]. GSDMD is the most common and best studied protein in pyroptosis. GSDMD is cleaved by caspase-1/4/5/11 to release its C and N-terminal fragments. The lipophilic N-terminal disrupts the cell membrane transition balance resulting in K+ outflow to the extracellular space and Na+ entry into the intracellular space by constituting pores in the cell membrane. As a result, the cell swells, the membrane ruptures, and the contents are released out of the cell, causing an intense inflammatory response with pyroptosis [15]. Previous studies have reported that pyroptosis may also cause a strong inflammatory response and clear or reduce cells through division, consistent with symptoms after SARS-CoV-2 infection. There is increasing evidence showing that pyroptosis may play a role in SARS-CoV-2 infection and its pathogenesis [15,29,30]. Xu et al. [18] investigated the relationship between pyroptosis and the severity of COVID-19. In this study, single-cell RNA-seq (scRNAseq) data of 37,607 immune system cells belonging to eight different cell types from four studies were analyzed. As a result of the study, it was determined that the expression of key markers of pyroptosis, such as IL-1β and IL18, was significantly higher in moderate and severe COVID-19 patients than in healthy volunteers. It was observed that caspase-1 was overexpressed in the spleen of hDPP4-Tg (human dipeptidyl peptidase 4 transgenic) mice infected with this virus and elevated levels of IL-1β in the serum. In addition to this, in this study, it was shown that the expression of caspase-1 and IL-1β decreased as a result of blockade of C5a-C5aR1 by an anti-C5aR1 antibody. Based on these data, it has been suggested that MERS-CoV infection induces overactivation of complement, which may contribute to pyroptosis and inflammation, and that C5aR1 may inhibit pyroptosis [31]. Chen et al [32] have stated that SARS-CoV Viroporin 3a activates the NLRP3 inflammasome, triggers IL-1β secretion in bone marrow-derived macrophages, and this suggested cell pyroptosis caused by SARS-CoV.

In our study, it was detected that there were no statistically significant differences between the biomarkers as a result of the mean comparison tests. However, it was observed that the IL-1β levels between groups of the control, the hospitalization, and the discharge were found to be remarkable: in the COVID-19 group, the IL-1β level at the discharge was higher than at the hospitalization; IL-1β levels of the COVID-19 group at both the hospitalization and the discharge periods were higher than the control group. Although statistically significant differences were not detected in our study, the higher IL-1β level in the patient group was considered as an immunological finding supporting the relationship between COVID-19 and pyroptosis. In addition, GSDMD, caspase-1, IL-1β and NLRP3 values were found to be higher in patients having high CRP at the hospitalization period. It was thought that this may be an important indicator of the parallelism between inflammation and pyroptosis. High levels of LDH are a general sign of tissue damage, which is supported by the widespread cell death of monocytes, alveolar epithelial cells, lung, and kidney endothelial cells. These cells are also competent to play a role in inflammasome activation and pyroptosis [33-38]. It has been shown that IL-1 family cytokines, LDH level and high GSDMD expression, which are considered as signs of pyroptosis in plasma, are associated with increasing risk of serious COVID-19 disease [38,39]. Kayagaki et al. [40] showed that IL-1β secretion decreased and release of LDH impaired as a result of blocking the GSDMD pathway in mice. In our study, the high IL-1β level in patients with high LDH level at the hospitalization period supports the relationship between pyroptosis and COVID-19, and in this respect, it is similar to the literature [41-45].

It has been shown that pyroptosis not only has an important role in infectious diseases, cardiovascular diseases, tumors, central nervous system diseases [39-43]. In addition, it has been shown that pyroptosis also plays an important role in the development of spontaneous inflammatory diseases, autoimmune diseases (systemic lupus erythematosus, inflammatory bowel diseases) and various metabolic diseases [46,47]. In our study, GSDMD, caspase-1, IL-1β and NLRP3 values have been found to be higher in patients with comorbidities both at the hospitalization and the discharge periods. This result is thought to be an important indicator that comorbid factors may affect pyroptosis.

Inflammation is induced by the inflammasomes. They have been linked to a number of inflammatory diseases. Our understanding of the processes by which the NLRP3 inflammasome is activated has significantly improved in light of recent evidence. Inflammasome involvement in the onset or progression of diseases with significant effects on public health, such as metabolic pathologies (obesity, type 2 diabetes, atherosclerosis), cardiovascular diseases (ischemic and non-

ischemic heart disease), inflammatory conditions (liver diseases, inflammatory bowel diseases, gut microbiome, rheumatoid arthritis), and neurologic disorders is also strongly supported by growing evidence in animal models and human studies [48]. In this study, the inadequacy of the study population, the heterogeneous distribution of measurements, and not studying of IL-18, another important cytokine released together with IL-1β in the pyroptosis mechanism, are the important limitations of our study.

Conclusion

Despite the decrease in the number of cases, the COVID-19 pandemic continues to maintain its importance. Different studies have been conducted on the etiopathogenesis, and pyroptosis, which is one of the cell death mechanisms, is one of them. In our study, IL-1β, which is one of the cytokines released as a result of cell death in the pyroptosis mechanism, was found to be higher in patients with a diagnosis of COVID-19 both at the hospitalization and the discharge compared to the control group. The high IL-1β level in patients with high LDH levels was another important finding supporting the relationship between pyroptosis and COVID-19. Studies on these pyroptosis-associated markers with larger patient population and different patient groups are needed.

Ethics Committee approval

Harran University, Medicine Faculty Ethics Committee was received the approval (29/11/2021, HRU/21.21.09). All procedures were performed in accordance with the Declaration of Helsinki.

Disclosures: There is no conflict of interest for all authors.

Acknowledgements: None.

Funding: None.

References

1. Rabi FA, Zoubi MS, Kasasbeh GA, Salameh DM, Al-Nasser AD. SARS-CoV-2 and coronavirus disease 2019: What we know so far. *Pathogens*. 2020;9:231. https://doi.org/10.3390/pathogens9030231
2. Republic of Turkey Ministry of Health, General Directorate of Public Health. COVID-19 (SARS-CoV-2 infection) general information, epidemiology and diagnosis. https://covid19.saglik.gov.tr/TR-66337/genel-bilgiler-epidemiyoloji-ve-tani.html. [Accessed 10 May 2022].
3. WHO Director-General's opening remarks at the media briefing on COVID-19 - 11 March 2020: World Health Organization; 2020. Available: https://www. who. int/ dg/ speeches/ detail/ who- director- general- s- openingremarks- at- the- media- briefing-on- covid- 19- 11- march- 2020 [Accessed 9 Apr 2020].
4. WHO Coronavirus (COVID-19) Dashboard. Available: https://covid19.who.int/ [Accessed 10 May 2022].
5. Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y, et al. Early Transmission Dynamics in Wuhan, China, of Novel Coronavirus Infected Pneumonia. *N Engl J Med.* 2020;382:1199. https://doi:10.1056/NEJMoa2001316
6. 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*. 2021;10:257. https://doi.org/10.3390/antiox10020257
7. Ardahanlı I, Akhan O, Aslan R, Celik M, Akyüz O. A new index in the follow-up of arrhythmia of Coronavirus Disease-2019 (COVID-19) patients receiving Hydroxychloroquine and Azithromycin therapy; index of cardiac electrophysiological balance. *Cumhuriyet Medical Journal.* 2021; 43(1):1-7. https://doi.org/10.7197/cmj.870158
8. Yıldırım AC, Alkan Cevıker S, Zeren S, Ekıcı MF, Yaylak F, Algın MC, Arık Ö. COVID-19 and related gastrointestinal symptoms: An observational study. *Marmara Medical Journal.* 2022; 35(2):244-248. https://doi.org/10.5472/marumj.1121879
9. Evlice O, Örs Şendoğan D, Ak Ö. Hemodializ Hastalarında COVID-19'un klinik seyri ve mortalite öngördürücüleri, tek merkez deneyimi. Biotech&Strategic Health Res. 2021; 5(2):105-112. https://doi.org/10.34084/bshr.929708
10. Dindar Demiray EK, Yılmaz M, Alıravcı ID, Alkan S. COVID-19-Akut Pankreatit İlişkisinin İncelenmesi. *İstanbul Gelişim Üniversitesi Sağlık Bilimleri Dergisi*. 2021; (13):130-143. https://doi.org/10.38079/igusabder.815768
11. Ardahanli I, Akhan O, Sahin E, Akgun O, Gurbanov, R. Myocardial performance index increases at long-term follow-up in patients with mild to moderate COVID-19. *Echocardiography*. 2022; 39(4):620-625. https://doi.org/10.1111/echo.15340
12. Üzümcügil AO, Demirkiran ND, Öner SK, Akkurt A, Alkan Çeviker S. Limb Ischemia Associated With Covid-19 and Its Treatment With Above-Knee Amputation. *Int J Low Extrem Wounds.* 2022;21(2):197-200. https://doi.org/10.1177/15347346211063257
13. Avcı E, Ardahanlı İ, Öztaş E, Dişibeyaz S. Is there a relationship between gastrointestinal symptoms and disease course and prognosis in COVID-19? A single-center pilot study. *The Turkish Journal of Academic Gastroenterology*. 2020; 19:103-108.
14. Alkan Çeviker S, Şener A, Yüksel C, Önder T, Akça A, Vurucu S, et al. Angioedema and acute urticaria in a patient with COVID 19 pneumonia: Favipiravir side effect or COVID-19 cutaneous manifestation. *Journal of Emergency Medicine Case Reports.* 2021; 12(2):65-67. https://doi.org/10.33706/jemcr.851107
15. Li JX, Chen LJ, Zhou CH, Bai F, Zhao Y, Zhang JG, et al. Insight to Proptosis in Viral Infectious Diseases. *Health* 2021;13:574-590. https://doi.org/10.4236/health.2021.135043
16. de Rivero Vaccari JC, Dietrich WD, Keane RW, de Rivero Vaccari JP. The Inflammasome in Times of COVID-19. *Front Immunol.* 2020; 11:583373. https://doi.org/10.3389/fimmu.2020.583373
17. Ferreira AC, Soares VC, de Azevedo-Quintanilha IG, Dias SDSG, Fintelman-Rodrigues N, Sacramento CQ, et al. SARS-CoV-2 engages inflammasome and proptosis in human primary monocytes. *Cell Death Discov*. 2021; 7(1):1-12. https://doi.org/10.1038/s41420-021-00428-w
18. Qian Xu, Yongjian Yang, Xiuren Zhang, James J. Cai. Association of proptosis and severeness of COVID-19 as revealed by integrated single-cell transcriptome data analysis. *İmmuno Informatics.* 2022; 6:100013. https://doi.org/10.1016/j.immuno.2022.100013

19. Republic of Turkey Ministry of Health, General Directorate of Public Health. COVID-19 (SARS-CoV-2 infection) Adult Patient Treatment. covid19.saglik.gov.tr/TR-66926/eriskin-hasta-tedavisi.html [Accessed 10 May 2022].
20. Frank D, Vince JE. Pyroptosis versus necroptosis: similarities, differences, and crosstalk. *Cell Death Differ.* 2019; 26(1):99-114. https://doi.org/10.1038/s41418-018-0212-6
21. Kerr JF, Wyllie AH, Currie AR. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer.* 1972; 26:239–257. https://doi.org/10.1038/bjc.1972.33
22. Lindqvist LM, Frank D, McArthur K, Dite TA, Lazarou M, Oakhill JS, et al. Autophagy induced during apoptosis degrades mitochondria and inhibits type I interferon secretion. *Cell Death Differ*. 2017; 25:782–794. https://doi.org/10.1038/s41418-017-0017-z
23. Segawa K, Nagata S. An apoptotic 'eat me' signal: phosphatidylserine exposure. *Trends Cell Biol.* 2015; 25:639–650. https://doi.org/10.1016/j.tcb.2015.08.003
24. White MJ, McArthur K, Metcalf D, Lane RM, Cambier JC, Herold MJ, et al. Apoptotic caspases suppress mtDNA-induced STING-mediated type I IFN production. *Cell.* 2014; 159:1549–1562. https://doi.org/10.1016/j.cell.2014.11.036
25. Menu P, Vince JE. The NLRP3 inflammasome in health and disease: the good, the bad and the ugly. *Clin Exp Immunol*. 2011; 166:1–15. https://doi.org/10.1111/j.1365-2249.2011.04440.x
26. Broz P, Dixit V. Inflammasomes: mechanism of assembly, regulation and signalling. *Nat Rev Immunol.* 2016;16:407-420. https://doi.org/10.1038/nri.2016.58
27. Yap JK, Moriyama M, Iwasaki A. Inflammasomes and Proptosis as Therapeutic Targets for COVID-19. *J Immunol.* 2020; 205(2):307-312. https://doi.org/10.4049/jimmunol.2000513
28. Chen S, Mei S, Luo Y, Wu H, Zhang J, Zhu J. Gasdermin Family: A Promising Therapeutic Target for Stroke. *Transl Stroke Res*. 2018; 9:555-563. https://doi.org/10.1007/s12975-018-0666-3
29. Jorgensen I, Rayamajhi M, Miao EA. Programmed Cell Death as a Defence against Infection. *Nat Rev Immunol.* 2017; 17:151-164. https://doi.org/10.1038/nri.2016.147
30. Soy M, Keser G, Atagunduz P, Tabak F, Atagunduz I, Kayhan S. Cytokine Storm in COVID-19: Pathogenesis and Overview of Anti-Inflammatory Agents Used in Treatment. *Clin Rheumatol.* 2020; 39:2085-2094. https://doi.org/10.1007/s10067-020-05190-5
31. Freeman TL, Swartz TH. Targeting the NLRP3 Inflammasome in Severe COVID-19. *Front Immunol.* 2020; 11:1518. https://doi.org/10.3389/fimmu.2020.01518
32. Jiang Y, Li J, Teng Y, Sun H, Tian G, He L, et al. Complement Receptor C5aR1 Inhibition Reduces Proptosis in hDPP4-Transgenic Mice Infected with MERS-CoV. *Viruses*. 2019; 11(1):39. https://doi.org/10.3390/v11010039
33. Chen IY, Moriyama M, Chang M, Ichinohe T. Severe Acute Respiratory Syndrome Coronavirus Viroporin 3a Activates the NLRP3 Inflammasome. *Front Microbiol*. 2019; 10:50. https://doi.org/10.3389/fmicb.2019.00050
34. Schurink B, Roos E, Radonic T, Barbe E, Bouman CS, de Boer HH, et al. Viral presence and immunopathology in patients with lethal COVID-19: a prospective autopsy cohort study. *Lancet Microbe.* 2020; 1(7):290–299. https://doi.org/10.1016/S2666-5247(20)30144-0
35. Varga Z, Flammer AJ, Steiger P, Haberecker M, Andermatt R, Zinkernagel AS, Moch H. Endothelial cell infection and endotheliitis in COVID-19. *Lancet*. 2020; 395(10234):1417–1418. https://doi.org/10.1016/S0140-6736(20)30937-5
36. Liu X, Lieberman J. A mechanistic understanding of proptosis: the fiery death triggered by invasive infection. Adv Immunol. 2017; 135:81-117. https://doi.org/10.1016/bs.ai.2017.02.002
37. Palazon- Riquelme P, Lopez- Castejon G. The inflammasomes, immune guardians at defence barriers. *Immunology*. 2018; 155(3):320–330. https://doi.org/10.1111/imm.12989
38. Bai B, Yang Y, Wang Q, Li M, Tian C, Liu Y. NLRP3 inflammasome in endothelial dysfunction. *Cell Death Dis.* 2020; 11:776. https://doi.org/10.1038/s41419-020-02985-x
39. Vora SM, Lieberman J, Wu H. Inflammasome activation at the crux of severe COVID-19. *Nat Rev Immunol.* 2021; 21:694–703. https://doi.org/10.1038/s41577-021-00588-x
40. Zhang J, Wu H, Yao X, Zhang D, Zhou Y, Fu B, et al. Pyroptotic macrophages stimulate the SARS-CoV-2-associated cytokine storm. *Cell Mol Immunol.* 2021; 18:1305–1307. https://doi.org/10.1038/s41423-021-00665-0
41. Kayagaki N, Stowe IB, Lee BL, O'Rourke K, Anderson K, Warming S, et al. Caspase-11 cleaves gasdermin D for non-canonical inflammasome signalling. *Nature*. 2015; 526:666–671. https://doi.org/10.1038/nature15541
42. Weihua Gong, Ying Shi, Jingjing Ren. Research progresses of molecular mechanism of proptosis and its related diseases. *Immunobiology*. 2020; 225(2):151884. https://doi.org/10.1016/j.imbio.2019.11.019
43. Marcin Dobaczewski, Wei Chen, Nikolaos G. Frangogiannis, Transforming growth factor (TGF)-β signaling in cardiac remodeling, *J Mol Cell Cardiol*. 2011;51(4):600-606. https://doi.org/10.1016/j.yjmcc.2010.10.033
44. Tan MS, Tan L, Jiang T. Amyloid-β induces NLRP1-dependent neuronal proptosis in models of Alzheimer's disease. Cell Death. 2014; 5:1382. https://doi.org/10.1038/cddis.2014.348
45. Jesus AA, Goldbach-Mansky R. IL-1 blockade in autoinflammatory syndromes. *Annu Rev Med.* 2014; 65(1):223-244. https://doi.org/10.1146/annurev-med-061512-150641
46. Luo B, Li B, Wang W, Liu X, Xia Y, Zhang C, et al. NLRP3 Gene Silencing Ameliorates Diabetic Cardiomyopathy in a Type 2 Diabetes Rat Model. *PLOS ONE*. 2014; 9(8):104771. https://doi.org/10.1371/journal.pone.0104771
47. Magna M, Pisetsky DS. The role of cell death in the pathogenesis of SLE: is proptosis the missing link?. *Scand J Immunol.* 2015; 82(3):218-224. https://doi.org/10.1111/sji.12335
48. Fusco R, Siracusa R, Genovese T, Cuzzocrea S, Di Paola R. Focus on the Role of NLRP3 Inflammasome in Diseases. *International Journal of Molecular Sciences.* 2020; 21(12):4223. https://doi.org/10.3390/ijms21124223