Differentially Expressed Inflammatory Cell Death-Related Genes and the Serum Levels of IL-6 are Determinants for Severity of Coronaviruses Diseases-2019 (COVID-19)

Mohammad Heidarvand[1], Reza Hosseini[1], Mohammad Kazemi[2], Alireza Andalib[1], Ramin Sami[3], Nahid Eskandari[1]*, Behrooz Ghezelbash[1]*

[1]Department of Immunology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran, [2]Department of Genetics, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran, [3]Department of Internal Medicine, School of Medicine, Khorshid Hospital, Isfahan University of Medical Sciences, Isfahan, Iran
*These two contributed equally as corresponding authors

Abstract

Background: Inflammatory cell death, PANoptosis, has been suggested to orchestrate the lymphocyte decrement among coronavirus disease-2019 (COVID-19) patients. The main aim of this study was to examine the differences in the expression of key genes related to inflammatory cell death and their correlation with lymphopenia in the mild and severe types of COVID-19 patients.

Materials and Methods: Eighty-eight patients (36 to 60 years old) with mild ($n = 44$) and severe ($n = 44$) types of COVID-19 were enrolled. The expression of key genes related to apoptosis (FAS-associated death domain protein, FADD), pyroptosis (ASC (apoptosis-associated speck-like protein containing caspase activation and recruitment domains (CARD)), the adapter protein ASC binds directly to caspase-1 and is critical for caspase-1 activation in response to a broad range of stimuli), and necroptosis (mixed lineage kinase domain-like, MLKL) genes were examined by reverse transcription-quantitative polymerase chain reaction (RT-qPCR) assay, and compared between the groups. The serum levels of interleukin (IL)-6 were measured by enzyme-linked immunosorbent assay (ELISA) assay.

Results: A major increase in the expression of FADD, ASC, and MLKL-related genes in the severe type of patients was compared to the mild type of patients. The serum levels of IL-6 similarly indicated a significant increase in the severe type of the patients. A significant negative correlation was detected between the three genes' expression and the levels of IL-6 with the lymphocyte counts in both types of COVID-19 patients.

Conclusion: Overall, the main regulated cell-death pathways are likely to be involved in lymphopenia in COVID-19 patients, and the expression levels of these genes could potentially predict the patients' outcome.

Keywords: Apoptosis, coronavirus disease-19, interleukin-6, lymphopenia, necroptosis, pyroptosis

INTRODUCTION

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), the reason for coronavirus disease-2019 (COVID-19), has affected millions of people throughout the world since its outbreak in December 2019, in Hubei Province, People's Republic of China.[1] Clinical manifestations of the disease varied considerably and ranged differently from fever and

Access this article online

Quick Response Code:

Website:
www.advbiores.net

DOI:
10.4103/abr.abr_232_22

How to cite this article: Heidarvand M, Hosseini R, Kazemi M, Andalib A, Sami R, Eskandari N, *et al.* Differentially expressed inflammatory cell death-related genes and the serum levels of IL-6 are determinants for severity of coronaviruses diseases-2019 (COVID-19). Adv Biomed Res 2023;12:102.

cough to acute respiratory distress syndrome (ARDS) and death.[2,3] Preclinical data revealed that the SARS-CoV-2 infection is associated with lymphopenia, as an important immunological abnormality, that has been linked to the disease severity and poor prognosis.[3] Although thanks to the vaccines, the disease spread is under control now, but understanding the molecular mechanisms underlying cell death and lymphopenia still is of high importance and would increase our knowledge about COVID-19 pathobiology and enable us to devise novel therapies in the future.[4,5]

To maintain hemostasis, there should be a balance between cellular proliferation, differentiation, and cell death.[5,6] However, in some conditions, including viral infections, this balance is disrupted through, for example, immune hyperactivation that favors cell death.[6] Previous studies have confirmed that programmed cell death is regulated tightly and could be operated through three interlinked mechanisms of apoptosis, pyroptosis, and necroptosis.[6] However, as mentioned earlier, dysregulated or uncontrolled cellular death is a feature of a wide variety of diseases, including viral infections.[7] In the case of apoptosis, there are two main pathways, extrinsic and intrinsic, that control its initiation.[8,9] The extrinsic pathway is induced by engaging death receptors (tumor necrosis factor (TNF)-alpha associated receptors) with their ligands that recruit adaptor protein FAS-associated death domain protein (FADD) as well as pro-caspase-8 and leads to the assembly of death-inducing signaling complex (DISC).[9,10] However, pyroptosis is dependent on caspase activity and is characterized by the activation of inflammasomes.[11] The event initiating the inflammasomes is the recognition of damage-/pathogen-associated molecular recognition patterns (DAMPs/PAMPs) by the cytosolic NOD-like receptors (NLRs).[11] The subsequent phosphorylation of NLRs recruits apoptosis-associated speck-like protein containing caspase activation and recruitment domains (CARD) (ASC) and finally, leads to the recruitment and activation of caspase-1.[11] Necroptosis is also another mechanism of cellular death in which the binding of human TNF to its cognate receptor (TNFRI) leads to the phosphorylation of the mixed lineage kinase domain-like (MLKL) that is then oligomerized and resulted in the formation of membrane pores.[12,13]

Strong evidence pinpoints that SARS-CoV-2 may also disrupt the function of the immune system by inducing PANoptosis.[14] Previous studies examining the role of apoptosis in dysfunctional immunity in COVID-19 patients have shown an up-regulation for several genes related to apoptosis.[14,15] Likewise, it has been shown that SARS-CoV-2 triggers necroptosis by enhancing the phosphorylation of the MLKL.[16] Noteworthy, shreds of evidence have shown that three major SARS-CoV-2 proteins, including envelope (En), and open reading frames (ORF8b) and ORF3a are involved in the inflammasomes activation and pyroptosis induction.[17,18]

Interleukin (IL-) 6 is a soluble mediator whose production of IL-6 leads to plasma levels of about 1–10 pg/ml[19,20] and

is also the major pro-inflammatory cytokine that controls the progress of immune responses and chronic inflammatory diseases.[21,22] Elevated serum IL-6 has been detected in patients with inflammatory diseases and the IL-6 levels correlate with disease activity.[23] Lymphopenia is a common observation in COVID-19 patients, and also higher levels of IL-6 in COVID-19 patients' sera is a feature of the disease that has widely been linked to lymphopenia.[23]

In COVID-19, there are possible causes of lymphopenia. In the whole body, in keeping immune homeostasis and also an inflammatory response, cells such as lymphocytes could show a key role. The mechanism of lymphopenia could be to provide a clear plan for the treatment of COVID-19. It suggests different mechanisms leading to reduce lymphocytes that include[24,25] the following: 1) viruses could infect lymphocytes, so lymphocytes death happens, and also these cells may be express the coronavirus receptor ACE2 as the main aim of viruses, 2) lymphatic organs such as lymph node might directly be destroyed by the virus. Dysfunction of cells and the damage of the coronavirus to organs could make acute lymphocyte failure. This must be proven by the pathological dissection in the upcoming, 3) lymphocyte apoptosis could happen by inflammatory cytokines. Lymphocyte deficiency could occur by TNFα and IL-6. The metabolic disorders via producing metabolic molecules could inhibit lymphocytes. Studies showed that lactic acid levels elevate in severe types of COVID-19 patients, which might inhibit the production of lymphocytes.[25,26] These different mechanisms might together cause lymphopenia, so more studies and research are looked for. Therefore, the main possible indicator of the severity and hospitalization in COVID-19 patients is lymphopenia.

Accordingly, this study aimed to examine the expression levels of the key genes involved in apoptosis, necroptosis, and pyroptosis in the mild and severe types of COVID-19 patients. The second aim was to examine the differences in the serum levels of IL-6 between the mild and severe types of COVID-19 patients. Likewise, the third was to examine the possible correlation between lymphopenia and gene expression, along with the serum levels of IL-6 in these groups of patients.

MATERIALS AND METHODS

Study design and patients
In this study, 88 patients diagnosed with the mild and severe types of COVID-19 aged, 36-60 years old were selected. The selection occurred in those, who were referred to the Khorshid Hospital of Isfahan city. Demographics of the patients were also obtained from their medical records and an additional questionnaire was employed to gather further information. The patients had no history of heart disease, diabetes mellitus, hypertension, immune deficiency, and/or autoimmune disease, and were selected among those who were referred to Khorshid Hospital in Isfahan city, Iran. The patients were involved voluntarily and informed about the study purposes

and procedures, and signed consent letters were obtained from them. The inclusion criteria for the mild type of disease were defined as those patients, who were detected with the COVID-19 polymerase chain reaction (PCR) test and had PO2 >94%. However, the exclusion criteria for the mild type of disease were a history of heart disease, diabetes mellitus, hypertension, immune deficiency, and/or autoimmune disease, shortness of breath, dyspnea, or abnormal chest imaging. The inclusion criteria for the severe type of disease were the positive PCR test together with the multiple symptoms including PO2 <94%, PaO2/FiO2 <300 mm Hg, respiratory rate >30 breaths/min, and lung infiltrates >50%. However, the exclusion criteria were the same as for the mild type of disease. In addition, the patients were willing to enroll in this study by signing the informed written consent. Demographics of the patients were obtained from their medical records and an additional questionnaire was employed to gather further information [Table 1].

Isolation of the peripheral blood mononuclear cells (PBMCs)

PBMCs were isolated by using the Ficoll-Hypaque ® gradient (Lymphodex, Inno-Train, F4375, CAS: 26873-85-8, Darmstadt, Germany) according to the manufacturer's instructions.

In brief, blood samples were taken and collected in EDTA-containing tubes, and were mixed with an equal volume of Ca^{2+}- and Mg^{2+}-depleted phosphate-buffered saline (PBS, pH 7.3). The mixture was gently layered on Ficoll-Hypaque solution in 15 ml conical tubes with the sample-to-Ficoll ratio at 2:1. Then, centrifugation was done at 2800 rpm for 25 min and the interphase (white layer) was collected as PBMCs. The obtained cells were washed with PBS twice and their count and viability were examined with a hemocytometer using trypan blue. Cells with at least 75% viability were used for the next experiments.

RNA extraction and reverse transcription-quantitative polymerase chain reaction (RT-qPCR) experiments

Total RNA was isolated from the PBMCs using the RNA Extraction Kit (Yekta tajhiz azma kit, Yekta tajhiz azma Co., Iran) according to the manufacturer's instructions.

Table 1: Primers used in qPCR experiments for the analysis of FADD, ASC, MLKL, and GAPDH gene-expression

Primer	Sequence
GAPDH-F	AAGCTCATTTCCTGGTATG
GAPDH-R	CTTCCTCTTGTGCTCTTG
FADD-F	ACTGTTGCGTTCTCCTTCTCT
FADD-R	GCTGGCTCGTCAGCTCAAA
ASC-F	GCTAACGTGCTGCGCGACAT
ASC-R	CCACTCAACGTTTGTGACCCT
MLKL-F	GTGGGAAAGAAGGTGGAAGAG
MLKL-R	GCCAAGGGTGATAATATGCTTC

Afterward, the quantity and quality of isolated RNA were determined by measuring the absorbance at 260 nm and 280 nm with a nanodrop 2000 (NanodropTM2000; Thermo Fisher Scientific, USA). Next, 5 μg of the total RNA was used to synthesize cDNA using the Bio fact 2X RT-q PCR Master Mix kit (BIB0012, Biofact Co. South Korea) and oligo-dT primers. The PCR primers used in this study were designed using the AlleleID 7.6 software (a pioneering tool that helps design oligos for strain differentiation microarrays) (PREMIER Biosoft International, USA) and synthesized by the Metabion GmbH (Planegg-Martinsried, Steinkirchen, Germany) [Table 1]. RT-qPCR reactions were performed at a total volume of 20 μl and the RT-qPCR was performed using the 2X SYBR Green RT-qPCR High ROX (Co. Pars Tous, Tehran, Iran) and the StepOne Plus™ RT-qPCR detection system (Sigma Aldrich, Germany). The PCR amplification conditions consisted of 15 min at 95°C followed by 45 cycles of denaturation step at 95°C for 20 s, annealing at 60°C for 30 s, and extension at 72°C for 30 s. To determine the melting temperature of the specific amplification products and primer, melting curve analysis was used. This research was accomplished in triplicate and repeated at least three times. Glyceraldehydes-3-phosphate dehydrogenase (GAPDH) was used as an endogenous control. The expression level of each target gene was calculated as 2−ΔΔCt [Table 1].

Enzyme-linked immunosorbent assay (ELISA)

Patients' sera were obtained by centrifuging blood samples at 4000 rpm for 6 min. At least,

100 μL of these sera were poured into microtubes and stored at -20°C in the freezer. For the analysis of IL-6 levels, ELISA assays were performed by a commercial kit (Co. Karmania Pars gene, Mashhad, Iran). The optical density (OD) values were obtained by measuring at 450 nm wavelength using the ELISA reader (Polar Star, Co.Labtech, Germany). Then, the serum levels of IL-6 were determined from ODs compared, to the standard curve built, based on the manufacturer's instruction.

Statistical analysis

Using SPSS, all of the statistical analysis was done (IBM Statistics Software V.25, IBM Co., USA). The GraphPad Prism version 5.0 was used to illustrate the figures and the results were expressed as the mean ± standard deviation (SD). The normality of data was assessed using the Kolmogorov–Smirnov test. Statistical comparisons using the Mann-Whitney test were used to compare the data sets. This study used the bivariate, Spearman for correlation. A *P* value of 0.05 or less was deemed statistically significant. All the protocols of this study were in accordance with the Helsinki declaration and confirmed by the Isfahan University of Medical Sciences Ethics Committee (IR.MUI.MED.REC.1399.1063).

RESULTS

This study investigated 88 COVID-19 patients consisting of 44 mild and 44 severe cases. In the severe cases, 23 (52.3%)

cases were males, and 21 (47.7%) cases were females, while in the mild cases, the ratio of males and females was equal. The mean age of the patients in both groups was 48 (ranged 36–60) years old and the distribution of age and gender in the two groups was insignificant ($P > 0.05$).

FADD, ASC, and MLKL genes expression analysis

The expression levels of three different genes involved in virus-induced apoptosis, necroptosis, and pyroptosis were examined in this study.

Significant changes were observed in the expression of the FADD, ASC, and MLKL genes between the mild and severe types of COVID-19 patients [Table 2 and Figure 1]. Likewise, a significant up-regulation of IL-6 levels in sera of severe types of COVID-19 patients was found compared to those with mild type.

The correlation of lymphopenia with the serum concentration of IL-6 and the expression levels of FADD, ASC, and MLKL

The results showed a significant linear correlation between the lymphopenia with the serum levels of IL-6 and the expression of FADD, ASC, and MLKL genes in the PBMCs of the mild and severe COVID-19 patients [$P < 0.05$, Table 3].

The FADD gene exhibited the most significant correlation with the status of lymphopenia among either the mild or severe types of COVID-19 patients in the bivariate correlation analysis.

Table 2: Comparative analysis of FADD, ASC, and MLKL genes expression in PBMCs, and the serum levels of IL-6 among mild and severe COVID-19 patients

Gene	Group	Cases (*n*)	Mean(±SD)	*P*
FADD	S	44	7.951 (1.405)	<0.001
	M	44	1.587 (3.014)	
ASC	S	44	7.286 (1.291)	<0.001
	M	44	2.294 (3.096)	
MLKL	S	44	10.822 (3.167)	<0.001
	M	44	2.764 (3.562)	
IL-6 (pg/ml)	S	44	45.227 (7.708)	<0.001
	M	44	18.954 (8.888)	
Lymphocyte	S	44	0.885 (0.060)	<0.001
	M	44	1.245 (0.054)	

Fas-associated protein with death domain (FADD); Apoptosis-associated speck-like protein containing a CARD (ASC); Mixed lineage kinase domain-like pseudokinase (MLKL); Interleukine-6 (IL-6); S: severe COVID-19, M: mild COVID-19

The R-values also showed a reverse interaction between the transcript levels of the investigated genes as well as IL-6 with the lymphocyte counts in the mild and severe types.

DISCUSSION

This study aimed to examine the expression levels of the key genes involved in apoptosis, necroptosis, and pyroptosis in the mild and severe types of COVID-19 patients. The second aim was to examine the differences in the serum levels of IL-6 between COVID-19 patients with the mild and severe types. Also, the third was to examine the possible correlation between lymphopenia with the gene expression as well as the serum levels of IL-6. Previous studies have suggested that several mechanisms including apoptosis, pyroptosis, and necroptosis may underlie the decrement of lymphocytes in COVID-19 patients.[3,14] On the other hand, increased IL-6, an important inflammatory cytokine, has also widely been shown to be related to the disease severity and lymphopenia in COVID-19.[27]

The results of this study showed that the expression levels of FADD, ASC, and MLKL genes related to apoptosis, pyroptosis, and necroptosis, respectively. In addition, these levels had a significant difference in the PBMCs isolated from the mild and severe types of COVID-19 patients.

The results also showed that there was a remarkable up-regulation in the expression of FADD, ASC, and MLKL genes in the severe type of patients, compared to the mild type ones. Similarly, a higher level of IL-6 was also detected in the serum of the COVID-19 patients, with the severe condition, by a significant difference, compared to the mild patients [Figure 1 and Table 2]. There was also a significant negative correlation between the transcript levels of the FADD, ASC, and MLKL genes, as well as the serum levels of IL-6 with the lymphocyte counts in COVID-19 patients with severe and mild conditions [Table 3].

The literature expresses that during the apoptosis, the binding of Fas-L (CD95L) to its cognate Fas receptor leads to the recruitment of adaptor protein of FADD and pro-caspase-8 that collect to form the DISC.[5] This complex mediates the later steps that lead to the activation of the executioner caspases-3, -6, and -7.[5]

Based on the key role of the FADD adaptor in the initiating of apoptosis, this study examined its gene expression in the

Table 3: The relationship between FADD, ASC, and MLKL expression, and serum levels of IL-6 with lymphopenia among mild and severe COVID-19 patients

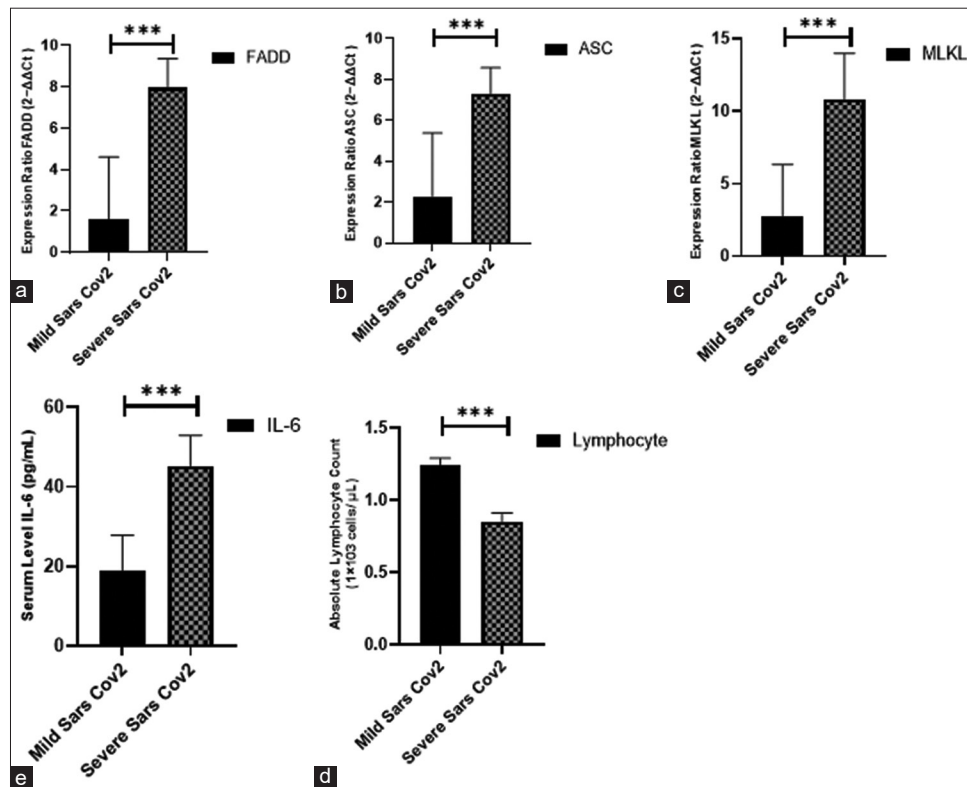| Group	*n*		Variables							
		Gene	FADD		ASC		MLKL		IL-6	
			R	*P*	*R*	*P*	*R*	*P*	*R*	*P*
Mild	44	Lymphocyte	-0.178	0.002	-0.268	0.001	-0.348	0.02	-0.019	0.004
Severe	44	Lymphocyte	-0.390	0.002	-0.265	0.02	-0.245	0.03	-0.119	0.002

Figure 1: The relative expression of FADD, ASC, and MLKL genes in the PBMCs, and the serum levels of IL-6 in the mild and severe types of COVID-19 cases. The fold changes of (a) FADD, (b) ASC, and (c) MLKL genes in PBMCs from mild and severe types of COVID-19 patients were examined by at least three different RT-qPCR experiments and were expressed as Mean ± SD. (e) The serum concentration of IL-6 between the mild and severe types of COVID-19 cases was examined by the ELISA experiments and was expressed as Mean ± SD, respectively (18.9 ± 8.9 pg/ml, 45.2 ± 7.7 pg/ml). (d) The differences in the absolute counts of the peripheral blood lymphocytes in two groups of COVID-19 patients (mild type vs severe type) were expressed as bar charts. * $P < 0.05$, **$P < 0.01$ and ***$P < 0.001$

PBMCs of the mild and severe types of patients. Likewise, this study found higher expression of the FADD gene in the severe type of COVID-19 patients, compared to the mild type ones, which is consistent with the higher lymphopenia, observed in these patients [Figure 1 and Table 2]. There is a study linking the extrinsic pathway of apoptosis to cellular damage and lymphopenia in COVID-19 patients.[9] Likewise, a previous study has demonstrated that the higher plasma levels of FasL in COVID-19 patients are directly associated with the apoptosis of T-cells.[28] Higher levels of CD95 (Fas) were also observed on the surface of T-cells from the intensive care unit (ICU) of the COVID-19 patients.[28] Collectively, these findings are proof of concept that binding FasL to Fas on T-cells could induce higher apoptosis, as present by the higher expression of the FADD gene in this study, leading to lymphopenia. However, on the contrary, a previous study has shown that the expression of the FADD gene is significantly lower in the severe type of COVID-19 patients, compared to those with the mild type of COVID-19.[15] We assume that the differences between the results of this study and those, which have been reported previously might be due to the discrepancy in the methodology. The patients enrolled in this study were those with the active disease (mild or severe type), however, the participants of the above-mentioned study were among those

who recovered from the disease. The higher levels of the FADD gene during the active disease in severe COVID-19 patients is a real-time viability of the apoptosis status, however, in the recovered patients, it is expected that changes in the genes expression (up-regulation/down-regulation) would be back to the normal levels. Therefore, the results of these two studies are complementary to each other, showing that during the active severe COVID-19, the gene levels, probably protein levels, of the FADD gene are up-regulated but, by the disease regression, its expression is decreased to its levels, compared to the mild or healthy type ones.

In spite of its activity in apoptosis, the FADD gene also plays a critical role during necroptosis, as another mechanism of regulated cellular death.[13] Indeed, by reviewing the literature, we found that the higher expression levels of the FADD gene not only be due to the higher apoptosis, but it is likely to be due to the higher necroptosis [Figure 1 and Table 2]. In support of this notion, a previous study examined the pathophysiology of COVID-19, and showed a significantly lower serum level of the FasL in COVID-19 patients, compared to the healthy donors.[29] Likewise, a significant negative correlation between the serum-FasL levels, neutrophil to the lymphocyte ratio (NLR), and the serum level of IL-6 were observed, suggesting that the serum-FasL, and therefore

extrinsic apoptotic pathway by the Fas/FasL axis, is not merely involved in lymphopenia in the COVID-19 patients.[29] On the other hand, similar to the results of this study, a significant up-regulation in the levels of MLKL pseudokinase, suggested the oligomerization of MLKL proteins downstream the FADD adaptor, which is defined as necroptosis, and might be the main mechanism of the regulated cell death and is responsible for the higher expression of the FADD gene.[29,30] In the same direction, a previous study has investigated the effects of TNF-α and interferon-gamma (IFN-γ) in the cellular damage, induced by SARS-CoV-2, and found that TNF-α and IFN-γ synergistically make inflammatory cell loss through the caspase-8 and FADD axis.[31] Indeed, it has been shown that the FADD deficiency protects against cell death induced by IFN-γ and TNF-α co-treatment independent of the intrinsic apoptosis pathway.[31] Although the results of this study showed that the MLKL gene expression was remarkably up-regulated in the PBMCs of the severe type of COVID-19 patients, but conflicting results have also been reported. In this regard, others have shown that although lymphopenia in COVID-19 patients correlates with caspase activation, but the transcripts of the MLKL are lower insignificantly in the CD8+ cytotoxic T-cells of the COVID-19 patients.[28] However, in the above-detailed report, the study did not mention whether the isolated T-cells are from the ICU of the COVID-19 patients or from the mild type of recovered patients.

Pyroptosis is another important pathway of regulated cell death that was considered in this study.[11] Noteworthy, the results also showed that the mRNA levels of the ASC,[32] a key regulator of inflammasome and caspase-1 activation, were up-regulated in the PBMCs of the severe type of the COVID-19 patients, compared to the mild type ones in a significant manner [Figure 1 and Table 2]. Although it is less known whether/how SARS-CoV-2 activates the nucleotide-binding oligomerization domain, leucine-rich repeat, and pyrin domain containing, which is a type of NOD-like receptor (NLRP3) and therefore, pyroptosis, but previous studies on the SARS-CoV-1 have shown that several viral proteins including E protein and ORF3a activate the NLRP3 via either triggering the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) signaling pathway to activate the transcription of some inflammatory chemokines and cytokines or by stimulating TNF receptor-associated factor (TRAF3)-mediated ubiquitously of ASC.[32]

Considering the high homology of ORF3a and E between SARS-CoV-1 and SARS-CoV-2, it can be inferred that the same, or at least, similar mechanisms, may occur during COVID-19.[33] This becomes more evident, as it has been shown that age-induced increased NLRP3/ASC mRNA expression, and thereby inflammasome over-activation is related to the lethality of the SARS-CoV-2.

The results also showed that the serum levels of IL-6 were expressively higher among the severe type of COVID-19 patients compared to mild type ones. Consistently,

one report has demonstrated that the serum levels of IL-6 are higher in severe types of patients and have increased levels during hospitalization.

The results also showed that the increase in IL-6 levels correlated with the disease deterioration and reached a peak at day 5 of hospitalization. Likewise, other studies have shown that elevated serum levels of IL-6 are closely associated with disease progression and outcome.[30]

Likewise, in this study, the correlation analysis of the mRNA levels of the FADD, ASC, and MLKL genes, along with the serum levels of IL-6 demonstrate a significant linkage between these variables and the lymphocyte counts. Therefore, the mRNA of mentioned genes, serum levels of IL-6, as well as peripheral blood lymphocyte counts could be utilized for the determination of disease prognosis [Table 3].

The strength point of this study is that few studies have examined the roles of PANoptosis in the induction of lymphopenia in COVID-19 patients; on the other hand, the contribution of these mechanisms in the mild or severe type of COVID-19 patients has not been examined yet. Nevertheless, the restriction of this study was the small number of under-investigated patients and it would be better generalized and concluded if it is more extensively investigated in the future.

Conclusion

Generally, the transcript levels of the FADD, ASC, and MLKL genes are up-regulated in the PBMCs of the severe type of COVID-19 patients, compared to those with the mild type of the disease. Furthermore, the serum levels of IL-6 increased drastically in the severe type of COVID-19 patients, compared to the mild type ones. Collectively, these results suggest that the three main pathways of regulated cell death, including apoptosis, necroptosis, and pyroptosis along with the increased serum levels of IL-6 are involved in the lymphopenia in the SARS-CoV-2 infection.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Hu B, Guo H, Zhou P, Shi ZL. Characteristics of SARS-CoV-2 and COVID-19. Nature Reviews Microbiology. 2021;19:141-54.
2. Heydari K, Rismantab S, Shamshirian A, Lotfi P, Shadmehri N, Houshmand P, *et al*. Clinical and paraclinical characteristics of COVID-19 patients: A systematic review and meta-analysis. medRxiv 2020.
3. Tan L, Wang Q, Zhang D, Ding J, Huang Q, Tang Y-Q, *et al*. Lymphopenia predicts disease severity of COVID-19: A descriptive and predictive study. Signal Transduct Target Ther 2020;5:1-3.
4. Sigal A. Milder disease with Omicron: Is it the virus or the pre-existing immunity? Nat Rev Immunol 2022;22:69-71.
5. Bertheloot D, Latz E, Franklin BS. Necroptosis, pyroptosis and apoptosis: An intricate game of cell death. Cell Mol Immunol 2021;18:1106-21.
6. Galluzzi L, Vitale I, Aaronson SA, Abrams JM, Adam D, Agostinis P, *et al*. Molecular mechanisms of cell death: Recommendations of the nomenclature committee on cell death 2018. Cell Death Differ 2018;25:486-541.
7. Nguyen LN, Kanneganti T-D. PANoptosis in viral infection: The missing puzzle piece in the cell death field. J Mol Biol 2022;434:167249.
8. Elmore S. Apoptosis: A review of programmed cell death. Toxicol Pathol 2007;35:495-516.
9. Donia A, Bokhari H. Apoptosis induced by SARS-CoV-2: Can we target it? Apoptosis 2021;26:7-8.
10. Thomson BJ. Viruses and apoptosis. Int J Exp Pathol 2001;82:65-76.
11. Man SM, Karki R, Kanneganti TD. Molecular mechanisms and functions of pyroptosis, inflammatory caspases and inflammasomes in infectious diseases. Immunol Rev 2017;277:61-75.
12. Orzalli MH, Kagan JC. Apoptosis and necroptosis as host defense strategies to prevent viral infection. Trend Cell Biol 2017;27:800-9.
13. Dhuriya YK, Sharma D. Necroptosis: A regulated inflammatory mode of cell death. J Neuroinflammation 2018;15:1-9.
14. Paolini A, Borella R, De Biasi S, Neroni A, Mattioli M, Lo Tartaro D, *et al*. Cell death in coronavirus infections: Uncovering its role during COVID-19. Cells 2021;10:1585.
15. Li S, Duan X, Li Y, Li M, Gao Y, Li T, *et al*. Differentially expressed immune response genes in COVID-19 patients based on disease severity. Aging (Albany NY) 2021;13:9265-76.
16. Li S, Zhang Y, Guan Z, Li H, Ye M, Chen X, *et al*. SARS-CoV-2 triggers inflammatory responses and cell death through caspase-8 activation. Signal Transduct Target Ther 2020;5:1-10.
17. Chen I-Y, Moriyama M, Chang M-F, Ichinohe T. Severe acute respiratory syndrome coronavirus viroporin 3a activates the NLRP3 inflammasome. Front Microbiol 2019;10:50.
18. Xu H, Akinyemi IA, Chitre SA, Loeb JC, Lednicky JA, McIntosh MT, *et al*. SARS-CoV-2 viroporin encoded by ORF3a triggers the NLRP3 inflammatory pathway. Virology 2022;568:13-22.
19. Akira S, Taga T, Kishimoto T. Interleukin-6 in biology and medicine. Adv Immunol 1993;54:1-78.
20. Matsuda T, Takahashi-Tezuka M, Fukada T, Okuyama Y, Fujitani Y, Tsukada S, *et al*. Association and activation of Btk and Tec tyrosine kinases by gp130, a signal transducer of the interleukin-6 family of cytokines. Blood 1995;85:627-33.
21. Yang B, Chang X, Huang J, Pan W, Si Z, Zhang C, *et al*. The role of IL-6/lymphocyte ratio in the peripheral blood of severe patients with COVID-19. Int Immunopharmacol 2021;97:107569.
22. Kishimoto T, Akira S, Narazaki M, Taga T. Interleukin-6 family of cytokines and gp130. Blood 1995;86:1243-54.
23. Tanaka T, Narazaki M, Kishimoto T. IL-6 in inflammation, immunity, and disease. Cold Spring Harb Perspect Biol 2014;6:a016295.
24. Zhou X, Ye G, Lv Y, Guo Y, Pan X, Li Y, *et al*. IL-6 drives T cell death to participate in lymphopenia in COVID-19. Int Immunopharmacol 2022;111:109132.
25. Tan L, Wang Q, Zhang D, Ding J, Huang Q, Tang YQ, *et al*. Lymphopenia predicts disease severity of COVID-19: A descriptive and predictive study. Signal Transduct Target Ther 2020;5:33.
26. Tan L, Wang Q, Zhang D, Ding J, Huang Q, Tang YQ, *et al*. Correction: Lymphopenia predicts disease severity of COVID-19: A descriptive and predictive study. Signal Transduct Target Ther 2020;5:61.
27. Jones SA, Hunter CA. Is IL-6 a key cytokine target for therapy in COVID-19? Nat Rev Immunol 2021;21:337-9.
28. André S, Picard M, Cezar R, Roux-Dalvai F, Alleaume-Butaux A, Soundaramourty C, *et al*. T cell apoptosis characterizes severe Covid-19 disease. Cell Death Differ 2022;29:1486-99.
29. Schweizer TA, Mairpady Shambat S, Vulin C, Hoeller S, Acevedo C, Huemer M, *et al*. Blunted sFasL signalling exacerbates TNF-driven neutrophil necroptosis in critically ill COVID-19 patients. Clin Transl Immunol 2021;10:e1357.
30. Ruskowski K, Neb H, Talbot SR, Choorapoikayil S, Adam EH, von Knethen A, *et al*. Persistently elevated plasma levels of RIPK3, MLKL, HMGB1, and RIPK1 in COVID-19 ICU patients. Am J Respir Cell Mol Biol 2022; 67:405-408.
31. Sun X, Liu Y, Huang Z, Xu W, Hu W, Yi L, *et al*. SARS-CoV-2 non-structural protein 6 triggers NLRP3-dependent pyroptosis by targeting ATP6AP1. Cell Death Differ 2022;29:1240-54.
32. Siu KL, Yuen KS, Castano-Rodriguez C, Ye ZW, Yeung ML, Fung SY, *et al*. Severe acute respiratory syndrome Coronavirus ORF3a protein activates the NLRP3 inflammasome by promoting TRAF3-dependent ubiquitination of ASC. FASEB J 2019;33:8865-77.
33. Yap JK, Moriyama M, Iwasaki A. Inflammasomes and pyroptosis as therapeutic targets for COVID-19. J Immunol 2020;205:307-12.