The evaluation of both the expression and serum protein levels of Caspase-8 and Mitogen-activated protein kinases one genes in patients with different degrees of SARS-CoV-2 infection

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Abstract

Aim: We performed the current study to evaluate the effects of caspase-8 (*CASP*8), Mitogen-activated protein kinase 1 (*MAPK*1) gene expression levels, and their products on preventing severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection.

Methods:40 individuals with SARS-CoV-2 infection were included in the current study. The patients were divided into four main groups mild (n=7), moderate (n=10), severe(n=14), and critical(n=9) levels of the disease. Individuals under the age of 18 and pregnant women were excluded. Patients were classified according to the WHO classification system (WHO/2019-nCoV/clinical/2021.1).

Results: Totally 40 individuals (male: 15 (37.5%); female: 25 (62.5%) with SARS-CoV2 infection were included in the current study. When all groups were taken into consideration, statistically significant differences were detected among all groups for both *CASP*82-ΔΔCt(p=0.006) and *MAPK1* 2-ΔΔCtvalues (p=0.015). Also, statistically significant differences were detected between mild and moderate (p=0.013), moderate and critical (p=0.018), and severe and critical (p=0.023) groups for lymphocytes.

Conclusions: The *CASP8/MAPK*1 expression levels and/or its products are essential in preventing injury caused by SARS-CoV-2 infection. They play crucial roles in maintaining cellular homeostasis and viability. Perhaps *CASP8/MAPK1* levels can provide information about the severity of the disease.

Introduction

The disease was named "Coronavirus Disease 2019 (COVID-19)" by the World Health Organization (WHO) in February after it was understood that the epidemic, which started with pneumonia of unknown etiology caused by a new coronavirus-related infection and associated with the seafood market in Wuhan, China, in December 2019 [1].

Coronaviruses are RNA viruses with rod-like protrusions on their surface and are enclosed, single-stranded, and positive-polarity. They are called coronaviruses because the protrusions mean 'corona,' which means 'crown' in Latin. Coronaviruses belong to the Coronaviridae family, Orthocoronavirinae subfamily, which is divided into four genera: alpha, beta, gamma, and deltacoronavirus [2].

Mitogen-activated protein kinases (*MAPK*s) may alter host defense and apoptotic mechanisms; they are a family of highly conserved serine-threonine protein kinases that link external stimuli, such as viral infections, to the cell nucleus and then to numerous outputs [3].

Because viruses ultimately depend on the host cell for their life cycle, the *MAPK* pathway is known to be active in viral infections [4]. In virus-infected cells, the *MAPK* cascade is also implicated in controlling immune response and apoptosis [5, 6]. Various DNA and RNA viruses use *MAPK* cascades to activate signal transduction pathways in infected host cells. To enhance their replication, they can use G-protein-mediated or external *MAPK* pathway activators such as tyrosine kinase receptors *MAPK* signaling can act as a positive or negative viral replication regulator. *MAPK* pathways can be triggered in the presence of both active and dormant viral cells [3, 7].

Extracellular Signal-Regulated Kinase 1/2 (ERK1/2), c-Jun NH2-terminal kinase (JNK), and p38 are all members of the *MAPK* cascade [8]. The development and evaluation of drugs that can target the *MAPK* pathway components to treat inflammatory and neurological illnesses, as well as cancer, has been going on for decades [9, 10].

Apoptosis and necroptosis (recently found controlled necrosis) are the most well-known forms of programmed cell death. The extrinsic (mitochondrial) pathway is triggered by the ligation of death receptors, a subset of the TNF receptor (TNFR) superfamily. The intrinsic (mitochondrial) pathway is also induced by ligating death receptors, a subset of the TNF receptor (TNFR) superfamily. The ligation of these death receptors can also cause necroptosis. The balance between the extrinsic apoptosis and necroptosis pathways affects whether cells live or die. *CASP*8 (aspartate-specific cysteine protease) is a critical regulator of programmed cell death activation.*CASP8* has been implicated in exogenous apoptosis induction and the inhibition of necroptosis [11, 12].

These findings reveal that caspase-8 can control inflammasome activation and the generation and maturation of pro-inflammatory cytokines IL1 and IL18 and so regulate inflammation in ways that are not dependent on death receptor signaling [11].

CASP8 and *MAPK1* have been studied in the context of viral infections [14–18]. This study aimed to figure out how SARS-CoV-2 infection works and what function *MAPK1* and *CASP8* play.

Methods

Patients and Groups

Totally 40 individuals (male:15(37.5%); female:25(62.5%)) with SARS-Covid19 infection were included in the current study.

Ethics

The study was approved by the Ministry of Health and Local Ethics Committee (Karabuk University Local Ethic Committee's approval document dated 11.11.2020 and numbered 338). The individuals with SARS-CoV-2 infection were divided into four main groups as mild (n = 7)], moderate(n = 10), severe(n = 14) and critical(n = 9) levels of the disease. Patients were classified according to the WHO classification system (WHO/2019-nCoV/clinical/2021.1). Individuals under 18 and pregnant women were excluded from the study.

Detection of Blood Parameters

The Beckman Coulter LH 780 Analyzer was used to examine the values of hemogram parameters [hemoglobin (HB), platelet (PLT), and lymphocyte]. The Roche Cobas Integra 400 plus auto analyzer was used to assess C-reactive protein (CRP) readings using original commercial kits.

The Cobas Roche t511 analyzer was used to measure D-Dimer levels. The serum LDH and ferritin levels were measured using a Roche Cobas 702 auto-analyzer.

Polymerase chain reactions were performed using SARS-CoV-2 RTqPCR Detection Kit (Bioeksen, Turkey) via a Real-Time PCR analyzer (Anatolia Gene works, Turkey).

Detection Of Human CASP8 and MAPK1 Concentrations via Enzyme-Linked Immunosorbent Assay (ELISA)

Both human *CASP8* and *MAPK1* levels were determined from blood serum samples of patients using the Human *CASP*8 (Invitrogen) (Catalog No: BMS2024) and *MAPK1* (Fine Test) (Catalog No: EH0665) kits via Enzyme-Linked Immunosorbent Assay (ELISA) method based on biotin double antibody sandwich technology. Bio-Tek Instruments ELX 50 Strip Washer was used as a microplate washer. Each well's optical density (OD value) was determined immediately using a 450 nm wavelength microplate reader (Bio-Tek Instruments ELX 800 Absorbance Microplate Reader). The findings of the samples were obtained by substituting the device's absorbance values in the calibration graph.

RNA isolation and cDNA synthesis

The patient's peripheral blood samples for RNA isolation were placed in RNase and DNase-free tubes containing ethylenediaminetetraacetic acid. Then, according to the manufacturer's instructions, RNA was extracted from peripheral blood samples of patients using RiboEx (Catalog No: 301-001) and Hybrid-R (Catalog No: 305 – 101) kits. Measurements were taken with a spectrophotometer to determine the adequacy of the RNA quality for the investigation. The extracted RNA was kept in RNase-free water at – 20°C. Using the A.B.T. cDNA Synthesis Kit (High Capacity) (Catalog No: C03-01-05), cDNA was obtained from isolated RNA. Mastermix used for cDNA synthesis include 2 µL RNA, 2 µL 10X RTase Reaction Buffer, 1µl dNTP mix, 3.5 µl RNase-Free Distilled Water, 1µl Reverse Transcriptase, 0.5µl RNase Inhibitor, and 2 µL Random Hexamer.

Relative gene expressions of CASP8 and MAPK1 Gene by Real-Time qPCR

The expression levels of *CASP8, MAPK1*, and the reference gene (*ACTB*) were detected via the Applied Biosystems 7500 and ViiA7 Real-Time PCR Systems for each cDNA sample of the patients. The A.B.T. 2X qPCR SYBR-Green Mastermix (Without Rox) (Catalog No: Q03-01-05) kit was used for the polymerase chain reactions (PCRs) in a final volume of 20 µL: 4 µL cDNA, 10 µL 2X MasterMix (with SYBR-Green), 3 µL RNase-Free Distilled Water, 1 µL QN ROX Reference Dye (Applied Biosystems cycler only), 1 µL Forward Primer (10 µM) and 1 µL Reverse Primer (10 µM). Cycle conditions of the Real-Time qPCR were Initial denaturation at 95^0C for 300 sec, 40 cycles of denaturation at 95^0C for 15 s, and 40 cycles of annealing at 60^0C for 60 seconds. *ACTB* transcript was used as a reference for quantitation of mRNA expressions and normalized according to the control group. Calculation of Fold Change had been calculated via processing $^{\Delta\Delta Ct}$ values as $^{2-\Delta\Delta Ct}$.

Statistical analysis

The data were analyzed via the Statistical Package for Social Sciences (IBM Corp., Armonk, NY, USA) for Windows 23.0. The Shapiro-Wilk test was used for the detection of data distribution. Because the data were not normally distributed ($P < 0.05$), non-parametric tests were used for statistical analysis. In addition to descriptive statistics [number, mean, standard deviation (SD)] for each variable, the Kruskal-Wallis test was

used to compare all groups; additionally, a polynomial regression test was carried out. The $p < 0.05$ was accepted as statistically significant.

Results

The mean age of patients was 63.225 ± 14.995. For age, no statistically significant differences were found among all groups (2 = 1.569, p = 0.666). Critical (63.16.248), severe (58.517.783), moderate (68.26), and mild (66.14314.893) were the ages of the groups, with critical (63.16.248), severe (58.517.783), moderate (68.26), and mild (66.14314.893) being the most common (Table 1).

Table 1

The hemogram values, *CASP8* $2^{-\Delta\Delta Ct}$, *MAPK1* $2^{-\Delta\Delta Ct}$, *CASP8* and *MAPK1* concentration levels of each group

	Groups				
	Mild (n = 7)	**Moderate (n = 10)**	**Severe (n = 14)**	**Critical (n = 9)**	**X^2/p**
Age (years)	66.143 ± 14.893	68 ± 8.26	58.5 ± 17.783	63 ± 16.248	1.569/0.666
HB	11.943 ± 1.885	12.46 ± 0.672	13.021 ± 2.113	12.522 ± 2.116	2.105/0.551
PLT	236.142 ± 103.798	251 ± 125.992	283.785 ± 106.610	236.888 ± 869.057	1.971/0.578
Lymphocyte	455.714 ± 297.706	1011 ± 481.582	755.714 ± 354.547	465.556 ± 305.414	11.641/**0.009***
CRP	157.973 ± 139.250	138.725 ± 114.137	139.037 ± 87.098	157.888 ± 106.882	0.354/0.950
Ferritin	474.829 ± 537.602	355.540 ± 235.059	474.664 ± 275.687	544.422 ± 392.461	1.956/0.582
D-dimer	1.121 ± 0.457	2.048 ± 2.037	2.134 ± 0.95	4.52 ± 6.407	8.913/**0.030***
LDH	410.571 ± 166.399	374.500 ± 181.785	349.857 ± 102.096	485.222 ± 162.243	4.160/0.245
CASP8 **$2^{-\Delta\Delta Ct}$**	313.959 ± 318.206	197.214 ± 236.022	88.097 ± 107.45	45.991 ± 60.256	12.413/**0.006***
CASP8 **conc.**	0.68 ± 0.698	0.547 ± 0.499	0.378 ± 0.273	0.228 ± 0.116	6.826/0.078
MAPK1 **$2^{-\Delta\Delta Ct}$**	9.1 ± 12.386	7.804 ± 6.589	3.321 ± 4.770	2.150 ± 3.834	10.429/**0.015***
MAPK1 **conc.**	22.953 ± 30.254	24.506 ± 34.85	14.341 ± 22.337	7.552 ± 1.479	3.249/0.355

CASP8: Caspase 8 MAPK1: Mitogen-Activated Protein Kinase 1 **Conc**: Concentration **HB**:Hemoglobin **PLT**: Platelet **CRP**: C-reactive protein **LDH**: Lactate dehydrogenase *: Statistically significant

The mean *CASP8* $2^{-\Delta\Delta Ct}$, *MAPK1* $2^{-\Delta\Delta Ct}$, *CASP8*, and *MAPK1* concentration levels of each group were given in Table 1, Fig. 1b,c, and Fig. 2b,c. When all groups were considered, statistically significant differences were detected among all groups for both *CASP8* $2^{-\Delta\Delta Ct}$ ($\chi 2 = 12.413$; $p = 0.006$) (Table 1) (Fig. 1b) and *MAPK1* $2^{-\Delta\Delta Ct}$ values ($\chi 2 = 10.429$; $p = 0.015$) (Table 1) (Fig. 2b). A binary comparison of the groups was performed to understand better the variations generated by which groups for *CASP8* $2^{-\Delta\Delta Ct}$. When the *CASP8* $2^{-\Delta\Delta Ct}$ values of the two groups were compared, statistically significant differences were found between mild and severe groups ($Z=-2.164$; $p = 0.030$), between mild and critical groups ($Z=-2.170$; $p = 0.030$), between moderate and severe groups ($Z=-2.284$; $p = 0.022$) and between moderate and critical groups ($Z=-2.613$; $p = 0.009$). However, there were no differences between mild and moderate ($Z=-1.464$; $p = 0.143$) and between severe and critical groups ($Z=-1.512$; $p = 0.131$) (Table 2).

Table 2

Binary comparison of the groups for *CASP8* $2^{-\Delta\Delta Ct}$, *MAPK1* $2^{-\Delta\Delta Ct}$, *CASP8* and *MAPK1* concentration and blood parameters

	Grps	Mild		Moderate		Severe		Critical	
CASP8		**Z**	**P**	**Z**	**P**	**Z**	**P**	**Z**	**P**
$2^{-\Delta\Delta Ct}$	**Mild**	-	-	-1.464	0.143	-2.164	**0.030***	-2.170	**0.030***
	Moderate	-1.464	0.143	-	-	-2.284	**0.022***	-2.613	**0.009***
	Severe	-2.164	**0.030***	-2.284	**0.022***	-	-	-1.512	0.131
	Critical	-2.170	**0.030***	-2.613	**0.009***	2.824	0.093	-	-
		Mild		**Moderate**		**Severe**		**Critical**	
		Z	**P**	**Z**	**P**	**Z**	**P**	**Z**	**P**
CASP8 **Conc**	**Mild**	-	-	-0.781	0.435	-1.419	0.136	-2.276	**0.023***
	Moderate	-0.781	0.435	-	-	-0.411	0.681	-1.922	**0.05***
	Severe	-1.419	0.136	-0.411	0.681	-	-	-1.577	0.115
	Critical	-2.276	**0.023***	-1.922	**0.05***	-1.577	0.115	-	-
		Mild		**Moderate**		**Severe**		**Critical**	
		Z	**P**	**Z**	**P**	**Z**	**P**	**Z**	**P**
MAPK1 $2^{-\Delta\Delta Ct}$	**Mild**	-	-	-0.586	0.558	-1.567	0.117	-2.170	**0.03***
	Moderate	-0.586	0.558	-	-	-2.197	0.028	-2.531	**0.011***
	Severe	-1.567	0.117	-2.197	0.028	-	-	-1.355	0.175
	Critical	-2.170	**0.03***	-2.531	**0.011***	-1.355	0.175	-	-
		Mild		**Moderate**		**Severe**		**Critical**	
		Z	**P**	**Z**	**P**	**Z**	**P**	**Z**	**P**
MAPK1 **Conc.**	**Mild**	-	-	-0.878	0.380	-1.492	0.136	-1.535	0.125
	Moderate	-0.878	0.380	-	-	-0.703	0.482	-0.980	0.327
	Severe	-1.492	0.136	-0.703	0.482	-	**-**	0.001	0.999
	Critical	-1.535	0.125	-0.980	0.327	0.001	0.999	-	-
		Mild		**Moderate**		**Severe**		**Critical**	
		Z	**P**	**Z**	**P**	**Z**	**P**	**Z**	**P**

CASP8: Caspase 8 MAPK1: Mitogen-Activated Protein Kinase 1 **Conc**: Concentration **Lymph**: Lymphocyte
*: Statistically significant

	Grps	Mild		Moderate		Severe		Critical	
D-dimer	**Mild**	-	-	-0.782	0.434	-2.164	**0.03***	-2.911	**0.004***
	Moderate	-0.782	0.434	-	-	-0.820	0.412	-1.633	0.102
	Severe	-2.164	**0.03***	-0.820	0.412	-	**-**	-1.323	0.186
	Critical	-2.911	**0.004***	-1.633	0.102	-1.323	0.186	-	-
		Mild		**Moderate**		**Severe**		**Critical**	
		Z	**P**	**Z**	**P**	**Z**	**P**	**Z**	**P**
Lymphocyte	**Mild**	-	-	-2.490	**0.013***	-1.830	0.067	-0.53	0.958
	Moderate	-2.490	**0.013***	-	-	-1.670	0.095	-2.370	**0.018***
	Severe	-1.830	0.067	-1.670	0.095	-	**-**	-2.269	**0.023***
	Critical	-0.53	0.958	-2.370	**0.018***	-2.269	**0.023***	**-**	-

CASP8: Caspase 8 MAPK1: Mitogen-Activated Protein Kinase 1 **Conc**: Concentration **Lymph**: Lymphocyte
*: Statistically significant

There were statistically significant variations in *CASP8* concentration between the mild and critical groups (Z=-2.276; p = 0.023) and between the moderate and critical groups (Z=-1.922; p = 0.05) when all groups were compared. (See Table 2).

A binary comparison of the groups was carried out to understand better which groups produced which variances for *MAPK1* $2^{-\Delta\Delta Ct}$. When the *MAPK1* $2^{-\Delta\Delta Ct}$ values of the two groups were evaluated, statistically significant differences were identified between mild and critical groups (Z=-2.170; p = 0.030), moderate and severe groups (Z=-2.197; p = 0.028), and moderate and critical groups (Z=-2.531; p = 0.011). When the *MAPK1* concentrations of all the groups were evaluated, there were no statistically significant differences between them ($\chi 2 = 3.249$; p = 0.355) (Table 2).

In Table 1, the hemogram values of each group are listed. When all groups were evaluated, statistically significant differences in D-dimer (p = 0.030) and lymphocyte (p = 0.009) were found (Table 1). Binary comparison of the groups was performed to determine which groups were responsible for the differences. According to this comparison, statistically significant differences were found between mild and severe (Z=-2.164, p = 0.030) and between mild and critical (Z=-2.911, p = 0.004) for D-dimer, respectively (Table 2).

The differences between mild and moderate (Z=-2.490; p = 0.013), moderate and critical (Z=-2.370; p = 0.018), and severe and critical (Z=-2.269, p = 0.023) lymphocytes were also statistically significant. (Table 2).

Discussion

In this study, we found a statistically significant difference for both *CASP8* $2^{-\Delta\Delta Ct}$, *and MAPK1* $2^{-\Delta\Delta Ct}$ values among all patient groups. In addition, *CASP8* concentrations are inversely related to illness severity in all study population groups, but the difference was not statistically significant among them. Although there was a statistically significant difference between mild and critical / moderate-critical patients in terms of *CASP8* $2^{-\Delta\Delta Ct}$, *CASP8* concentration, and *MAPK1* $2^{-\Delta\Delta Ct}$ levels, no difference was detected in *MAPK1* concentration between the whole study population. Also; we found a significant difference between the groups for D-dimer and lymphocyte values, which are among the poor prognostic factors (LDH, ferritin, D-dimer, hemoglobin, lymphocyte, CRP) and used clinically for SARS-CoV-2 infection.

It was reported that as HB [19–20] and lymphocyte [21] levels decrease, LDH [22, 23], D-dimer [24–26], ferritin [27, 28] levels increase and disease severity increases. Similarly, our study found that the severity of the disease increased, as the D-dimer level, which is one of the poor prognostic factors used in the clinic, increased depending on the SARS-CoV-2 infection among the groups. Deepening Lymphopenia was detected as the disease got severe. The main reason we could not find a statistical significance between CRP and ferritin values, which are the indicators of inflammation, may be caused by severe and critical patients taking anti-inflammatory treatments such as prednisolone, an IL-1 and IL-6 antagonist.

Because *MAPK*s are heterogeneous kinases that can phosphorylate serine and threonine residues in a variety of proteins, the formation of *MAPK*-associated pathways is thought to be the most likely cause of significant pro-inflammatory cytokine production (activation of the host's innate response) [29, 30].

Tiwari et al. designed an experimental study to describe the viral pathogenesis mechanism associated with complement and coagulation pathways in SARS-CoV-2 infection [30]. Protein-protein interactome profile was initially constructed based on the structural similarities of viral and host proteins; advanced computational analysis combined with Gene Oy (ntologGO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis suggest pathways linked with viral pathogenesis that have been annotated. Analyzes including *MAPK* signaling, complement and coagulation cascades, endocytosis, PD-L1 expression, PD-1 checkpoint pathway in cancer, and C-type lectin receptor signaling pathways were all performed. The GO analysis of these proteins discovered the innate immune system, cytokine signaling, *MAPK* signaling pathway, neutrophil degranulation, complement, and coagulation stages. They discovered that the *MAPK1, MAPK3*, AKT1, and SRC genes are all important in these pathways and have maximum overlap. As a result, they hypothesized that the *MAPK* signaling pathway is essential for the innate immune response to SARS-CoV-2 infection and the pathophysiology that underpins it [30].

In our study, when a pairwise comparison was performed in terms of *MAPK*1 $2^{-\Delta\Delta Ct}$ values, statistically significant differences were found between mild and critical groups (Z=-2.170; p = 0.030), between moderate and severe groups (Z=-2.197; p = 0.028), between moderate and critical groups (Z=-2.531; p = 0.011). There were no statistically significant differences when all groups were compared in terms of *MAPK*1 concentration.

Patients infected with SARS-CoV-2 have a severe inflammatory reaction to the virus and various systemic symptoms linked to the release of high amounts of pro-inflammatory cytokines (cytokine storms) [31, 32]. One proposed relationship between cytokine storm and organ damage is the cell death process. Pyroptosis,

apoptosis, and necroptosis are the most well-known programmed cell death mechanisms [33]. Pyroptosis and necroptosis are inflammatory cell death methods that release cytokines and other cellular components to trigger inflammation and alert immune cells to pathogenic or sterile damage, whereas apoptosis is considered immune-silent. [32]. Caspase-3 and − 7 carry out apoptosis following the activation of upstream initiator caspases caspase-8/10 or -9 [34, 35].

In COVID-19 patients, Karki et al. investigated the impact of substantially elevated pro-inflammatory cytokines on inflammatory cell death, inflammation, tissue and organ damage, and mortality [32]. The *MAPK* pathway, which regulates the expression of cytokines and chemokines, promotes the formation of cytokine storms. The type I interferon (IFN-I), *MAPK*, and ferroptosis pathways were activated while the disease was active, and they gradually recovered as the patient's health improved [36].

In the apoptosis process, *CASP8* plays the initiator role, whereas *CASP3* and 6 play the executioner role. In lung epithelial cells, SARS-CoV-2 infection activates caspase-8, resulting in cell death and inflammatory cytokine processing. The stimulation of virus-induced apoptosis, necroptosis, and inflammation in the lungs of a SARS-CoV-2-infected HFH4-hACE2 transgenic mouse model was also investigated (a valid model for studying SARS-CoV-2 pathogenesis). The analysis of postmortem lung sections of fatal COVID-19 patients revealed not only massive inflammatory cell infiltration, necrotic cell debris, and pulmonary interstitial fibrosis but also apoptosis and necroptosis [37]. Gulhan et al. [38] investigated the link between *CASP3* and the severity of COVID-19 infection and discovered a significant difference in *CASP3* $2^{-\Delta\Delta Ct}$ levels among all groups in a disease severity classification ($p = 0.014$). *CASP3* $2^{-\Delta\Delta Ct}$ values discovered statistically significant differences between mild and critical groups ($p = 0.003$) and intermediate and critical groups ($p = 0.009$). Statistically significant changes in *CASP3* concentration values between mild and severe groups ($p = 0.030$) and between mild and critical groups were also discovered.($p = 0.012$) [36].

In our study, we concluded that when both *CASP8* $2^{-\Delta\Delta Ct}$ **and** *CASP8* concentration decreased, the severity of the disease increased. Also, we detected a significant relationship between *CASP8* $2^{-\Delta\Delta Ct}$ level and disease severity, and statistically significant differences were detected among all groups for *CASP8* $2^{-\Delta\Delta Ct}$ ($p = 0.006$). There were statistically significant differences between mild and critical groups ($p = 0.030$), moderate and critical groups ($p = 0.009$), moderate and severe groups ($p = 0.022$), and mild and severe groups ($p = 0.030$) when the two groups were compared in terms of *CASP8* $2^{-\Delta\Delta Ct}$.

CASP8 levels may offer information about the severity of SARS-CoV-2. *CASP8* gene expression begins to rise when the virus is encountered. The severity of the illness probably decreases when the *CASP8* and *MAPK1* gene expression capacity increase.

CASP8 and *MAPK1* gene expression and/or products may change depending on the cellular damage caused by SARS-CoV-2 infection. This cycle can be repeated until the system reaches equilibrium. *CASP8* and *MAPK1* expression levels should not be fewer than those required to trigger and maintain apoptosis, and they should not be high enough to cause a cytokine storm. This should be at a level that keeps the body in a state of homeostasis.

After SARS-CoV-2 infection, it is logical to say that the *CASP8/MAPK* gene and/or its products play a role in preventing damage and maintaining cellular homeostasis and viability. *CASP8/MAPK* levels can serve as an indicator of COVID-19 disease. The discovery of disease pathology processes during COVID-19 infection may pave the way for developing evidence-based treatment solutions to address this public health problem. More research on the activity levels of *CASP8* and *MAPK1* is needed to understand the current condition better.

Declarations

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Conflict of interest:

All author state that there is no conflict of interest.

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Figures

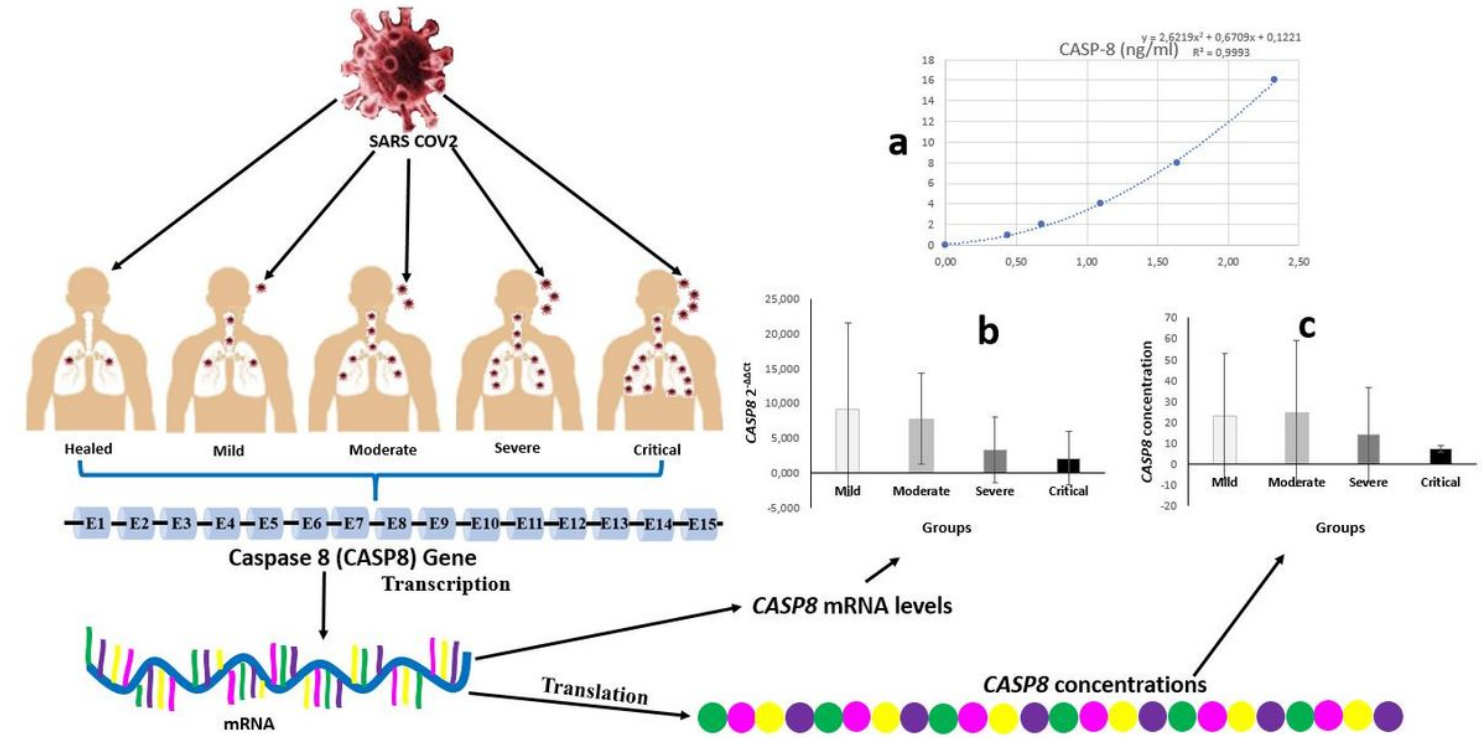

Figure 1

Demonstrative examples of SARS-CoV-2 infection. The patients were divided into four main groups as mild, moderate, severe and critical. Both *CASP8* 2-ΔΔCt levels and *CASP8* concentration levels of patients were detected. SARS-CoV-2 infection caused cellular damage and a response occurs in the nucleus of cells. *CASP8* gene is transcribed *CASP8* mRNA and *CASP8* proteins are translated from mature *CASP8* mRNA. ELISA standard curve given in figure 1 (a). Statistically significant differences were detected among all groups for *CASP8* 2-ΔΔCtlevels (b). Caspase 8 serum concentration levels of groups was given in figure 1 (c).

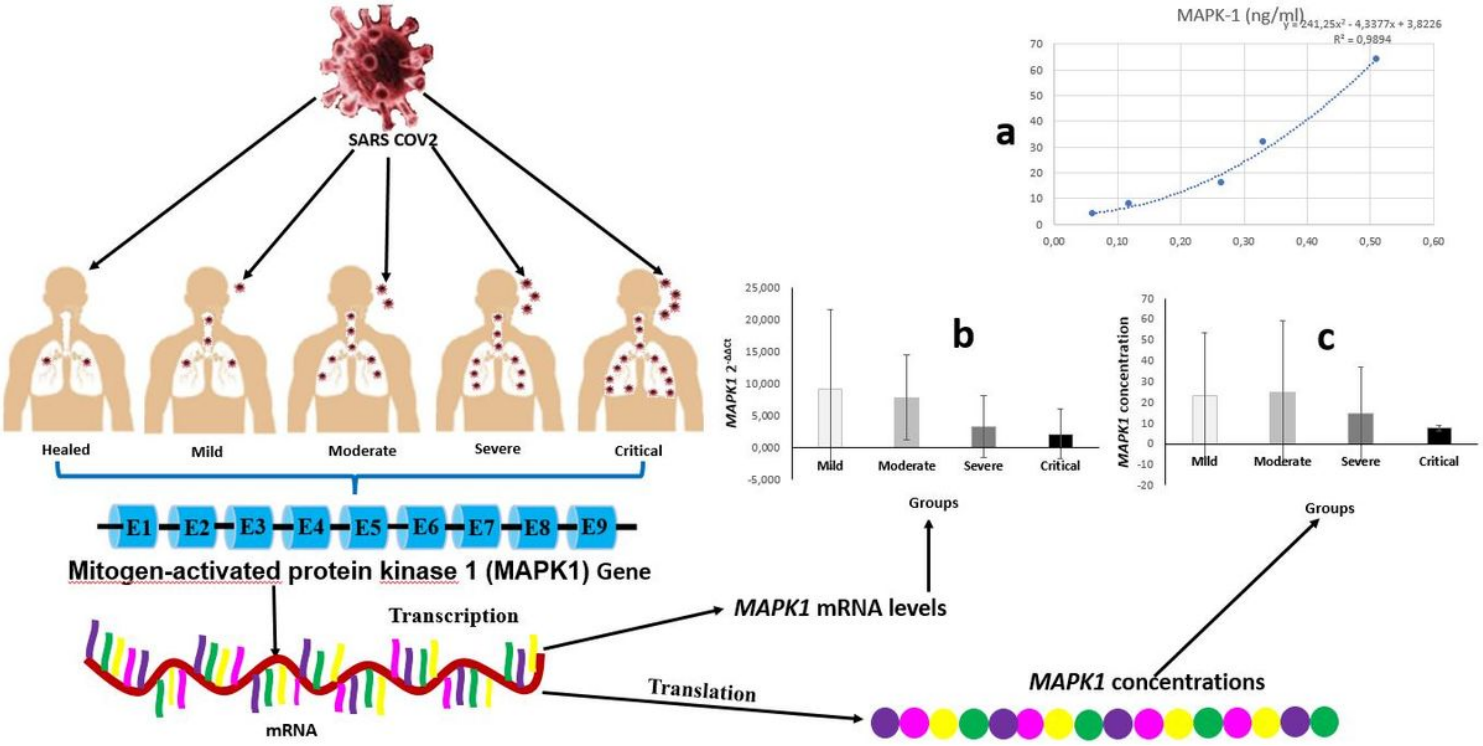

Figure 2

Demonstrative examples of SARS-CoV-2 infectionThe patients were divided into four main groups as mild, moderate, severe and critical. Both *MAPK1* 2-ΔΔCt levels and *MAPK1* concentration levels of patients were detected. SARS-CoV-2 infection caused cellular damage and a response occurs in the nucleus of cells. *MAPK1* gene is transcribed *MAPK1*mRNA and *MAPK1* proteins are translated from mature *MAPK1* mRNA. ELISA standard curve was given in figure 1(a). Statistically significant differences were detected among all groups for *MAPK1* 2-ΔΔCtlevels (b). *MAPK*1 serum concentration levels of groups given in figure 1(c).

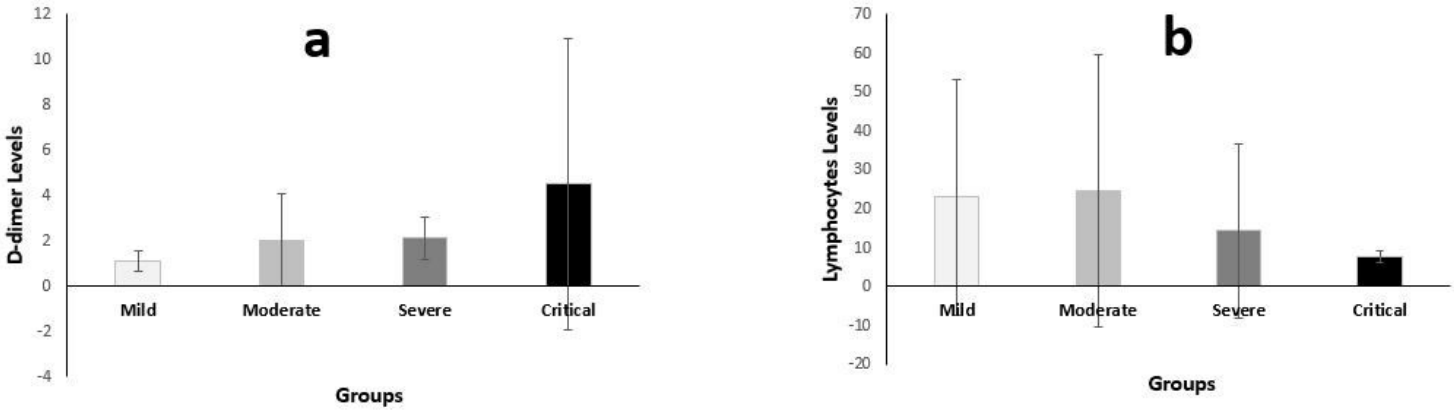

Figure 3

D-dimer (a) and lymphocytes(b) levels of groups