

## RESEARCH ARTICLE

# The evaluation of both the expression and serum protein levels of Caspase-3 gene in patients with different degrees of SARS-CoV2 infection

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## Abstract

To evaluate the effects of Caspase-3 (CASP3) gene expression and serum levels on preventing severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. A total of 41 individuals (male: 21; female: 20) with SARS-CoV-2 infection were included in the current study. Hemograms were examined from patient blood samples, and CASP3 gene expression levels were detected. Also, human CASP3 levels were determined from the serum samples of patients. The mean age of patients was  $56.220 \pm 18.937$  years. Significant differences were detected among all groups for CASP3  $2^{-\Delta\Delta C_t}$  ( $p = 0.014$ ) and CASP3 concentration ( $p = 0.024$ ). The relationship between CASP3  $2^{-\Delta\Delta C_t}$  levels and hemoglobin ( $p = 0.023$ ), between CASP3  $2^{-\Delta\Delta C_t}$  levels and C-reactive protein (CRP) ( $p = 0.001$ ), between CASP3  $2^{-\Delta\Delta C_t}$  levels and ferritin ( $p = 0.003$ ), between CASP3  $2^{-\Delta\Delta C_t}$  levels and lactate dehydrogenase ( $p = 0.001$ ), and between CASP3  $2^{-\Delta\Delta C_t}$  levels and SpO<sub>2</sub> ( $p = 0.006$ ) were statistically significant. Also, the relationship between CASP3 concentration levels and SpO<sub>2</sub> was statistically significant ( $p < 0.046$ ). The CASP3 gene and/or its products have an important function to prevent injury caused by SARS-CoV-2 infection. They play crucial roles in maintaining cellular homeostasis and viability. Perhaps CASP3 levels may provide information about the severity of the disease.

## KEYWORDS

CASP3 gene, cellular homeostasis, SARS-CoV-2

## 1 | INTRODUCTION

Coronavirus disease 2019 (COVID-19) is an infectious disease that primarily affects the lungs caused by a novel betacoronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It was first described in December 2019 in China and was later declared the cause of the pandemic. The novel virus was named SARS-CoV-2 by World Health Organization (WHO). SARS-CoV-2 is more than 79% homologous with SARS-CoV. Despite the genetic sequencing similarity of the novel coronavirus with the SARS virus, managing this unknown disease with its nonspecific manifestations remains challenging for physicians.<sup>1,2</sup>

The cysteinyl aspartate-specific proteinase-3 (CASP3) gene, located at chromosomal 4q35.1 region and including nine exons, is a common key enzyme in the apoptosis and pyroptosis pathways. This enzyme belongs to the caspase (from cysteine-aspartate protease) family, one of the six protease families with essential functions in normal cellular development.<sup>3,4</sup>

Initiation of apoptosis occurs both intrinsic or extrinsic pathway. As initiator caspases, caspases-8 and -10 are activated and they can then directly cleave and activate effector caspases, such as CASP3. As a result, apoptosis occurs.<sup>5</sup> It was reported that compared with the control group, lipopolysaccharide-induced oxidation and

apoptosis cause active CASP3 overexpression and cell viability inhibition.<sup>6</sup> As a result, apoptosis increases due to the increase in CASP3 level. We planned the current study with the thought that to eliminate potentially harmful cells damaged by SARS-CoV-2 infection for the organism in patients with different disease severity classification, the rate of apoptosis will increase depending on the elevation of both caspase 3 mRNA and CASP3 serum levels, so the virulence and severity of the disease will decrease by the elimination of virus-infected cells.

Both past and present studies about the relationship between the CASP3 gene and various viral infections, such as Enterovirus 71 (EV71),<sup>7-9</sup> parvovirus H1,<sup>10</sup> human cytomegalovirus,<sup>11</sup> hepatitis C virus,<sup>12</sup> H5N1 Cambodia PA virus,<sup>13</sup> Caprine parainfluenza virus type 3,<sup>14</sup> and others have been performed. However, to the best of our knowledge, no studies have been conducted about the expression levels and functions of nucleolin on CASP3 gene expression in patients with different degrees of cellular injury caused by SARS-CoV-2 infection who had mild illness and healed from the disease and those with mild disease, moderate, severe, and critical illness. Thus, we performed the current study to evaluate the effects of CASP3 gene expression and serum levels on preventing SARS-CoV-2 infection. The current study provides the initial insights into the putative role of the CASP3 in SARS-CoV-2 infection.

## 2 | MATERIALS AND METHODS

### 2.1 | Patients and groups

Totally 41 individuals (male: 21; female: 20) with SARS-CoV2 infection were included in the current study. The study was approved by both the Ministry of Health and Local Ethics Committee (Duzce University Local Ethic Committee's approval document dated 11.05.2020 and numbered 90). The patients were divided into four main groups such as mild (both patients with mild illness and healed from the disease [ $n = 8$ ], and patients with mild disease [ $n = 8$ ]), moderate ( $n = 8$ ), severe ( $n = 9$ ), and critical ( $n = 8$ ). Under 18 age and pregnant women were excluded. Patients were classified according to the <https://www.covid19treatmentguidelines.nih.gov/overview/clinical-spectrum/>.

**Mild illness:** Individuals who have any of the various signs and symptoms of COVID-19 (e.g., fever, cough, sore throat, malaise, headache, muscle pain, nausea, vomiting, diarrhea, loss of taste, and smell) but who do not have shortness of breath, dyspnea, or abnormal chest imaging.

**Moderate illness:** Individuals who show evidence of lower respiratory disease during clinical assessment or imaging and who have an oxygen saturation ( $\text{SpO}_2$ )  $\geq 94\%$  on room air at sea level.

**Severe illness:** Individuals who have  $\text{SpO}_2 < 94\%$  on room air at sea level, a ratio of arterial partial pressure of oxygen to fraction of inspired oxygen ( $\text{PaO}_2/\text{FiO}_2$ )  $< 300$  mmHg, respiratory frequency  $> 30$  breaths/min, or lung infiltrates  $> 50\%$ .

**Critical illness:** Individuals who have respiratory failure, septic shock, and/or multiple organ dysfunction.

Patients with mild disease and a duration of disease at least 1 month were defined as "healed."

#### 2.1.1 | Detection of blood parameters

The blood samples of hemogram parameter (hemoglobin [HB], platelet [PLT], lymphocyte) values were analyzed in the Beckman Coulter LH 780 Analyser. C-reactive protein (CRP) values measurements were performed by using original commercial kits in the Roche Cobas integra 400 plus auto analyzer. D-Dimer levels were analyzed by analyzer of Cobas Roche t511. Serum lactate dehydrogenase (LDH), ferritin levels were analyzed by auto-analyzer of Roche Cobas 702.

Polymerase chain reactions were performed in Real-Time PCR analyzer (Anatolia Geneworks) and SARS-CoV-2 RTqPCR Detection Kit (Bioeksen) was used for these analyzes.

#### 2.1.2 | Blood collection

The blood samples from the patients were taken. Hemogram was examined from the blood samples of patients and also CASP3 gene expressions levels were detected. Additionally, Human Caspase 3 level was determined from the serum samples obtained from the blood of the patients.

#### 2.1.3 | Detection of human Caspase 3 concentration via enzyme-linked immunosorbent assay (ELISA)

Human Caspase 3 level was determined from human serum using the Human Caspase 3 kit (Bioassay Technology) (Catalog No: E4804Hu) via ELISA method based on biotin double antibody sandwich technology. Bio-Tek Instrumens ELX 50 Strip Washer was used as microplate washer. Determination of the optical density (OD value) of each well was performed immediately using a microplate reader (Bio-Tek Instrumens ELX 800 Absorbance Microplate Reader) at 450 nm wavelength. The results of the samples were obtained by replacing the absorbance values taken from the device in the calibration graph.

#### 2.1.4 | RNA isolation and complementary DNA (cDNA) synthesis

Whole blood samples of patients were obtained in RNase and DNase free tubes containing ethylenediaminetetraacetic acid for RNA isolation. Then RNA was isolated from the whole blood samples of the patients using QIAamp RNA Blood Mini isolation kits (Catalog No: 52304) according to the manufacturer's instructions. Measurements were made in a spectrophotometer to evaluate the suitability of the RNA quality for the study. The isolated RNA was stored at  $-20^\circ\text{C}$  in RNase-free water until the study time. Using the QuantiTect<sup>®</sup>

Reverse Transcription cDNA synthesis kit (Catalog No: 205311), cDNA was obtained from isolated RNA.

### 2.1.5 | Relative gene expressions of CASP3 Gene by real-time qPCR

For each cDNA sample, the expression levels of CASP3 and the reference gene (ACTB) were analyzed using the Applied Biosystems 7500 and ViiA7 Real-Time PCR Systems. Polymerase chain reactions (PCRs) were in a final volume of 20  $\mu$ l: 4  $\mu$ l cDNA, 3  $\mu$ l RNase-Free Distilled Water, 10  $\mu$ l 2x QuantiNova SYBR Green PCR Master Mix (Catalog No: 208052), 1  $\mu$ l QN ROX Reference Dye (Applied Biosystems cyclor only) and 2  $\mu$ l primer-probe set (Oligomer). Cycle conditions of the Real-Time qPCR were initial denaturation at 95°C for 120 s, 40 cycles of denaturation at 95°C for 5 s, and 40 cycles of annealing at 60°C for 10 s. ACTB transcript is used as reference for quantitation of mRNA expressions and normalized according to the control group. Calculation of fold change had been made by processing  $\Delta\Delta C_t$  values as  $2^{-\Delta\Delta C_t}$ .

## 2.2 | Statistical analysis

The data were evaluated using the Statistical Package for Social Sciences (IBM Corp.) for Windows 23.0. The Shapiro–Wilk test was used to detect the distribution of data. As the data were not normally distributed ( $p < 0.05$ ), nonparametric tests were used for statistical analysis. In addition to descriptive statistic (number, mean, standard deviation [SD]) for each variable, Kruskal–Wallis test was used for the comparison of all groups, also polynomial regression test was

performed. The  $p$  value lower than 0.05 ( $p < 0.05$ ) was accepted as statistically significant.

## 3 | RESULTS

Totally 41 individuals (male: 21; female:20) with SARS-CoV2 infection were included in the current study. The mean age of patients were  $56.220 \pm 18.937$ . Statistically significant differences were detected among all groups for age of patients ( $\chi^2 = 20.210$ ,  $p < 0.0001$ ). The ages of the individuals included in the study were critical ( $71.875 \pm 12.264$ ), severe ( $70.375 \pm 12.247$ ), moderate ( $58.222 \pm 11.355$ ), and mild ( $40.188 \pm 15.272$ ) from the oldest to the youngest, respectively (Table 1).

The mean CASP3  $2^{-\Delta\Delta C_t}$  and CASP3 concentration values of each group are given in the Table 1. When the all groups were considered, statistically significant differences were detected among all groups for CASP3  $2^{-\Delta\Delta C_t}$  ( $\chi^2 = 10.609$ ;  $p = 0.014$ ) (Table 1) (Figure 1A) and CASP3 concentration ( $\chi^2 = 9.427$ ;  $p = 0.024$ ) (Table 1) (Figure 1C). To understand which groups causes these differences, double comparison of the groups was performed. When the two groups were compared in terms of CASP3  $2^{-\Delta\Delta C_t}$  values, there were statistically significant differences between mild and critical groups ( $\chi^2 = 8.825$ ,  $p = 0.003$ ) and between moderate and critical groups ( $\chi^2 = 6.750$ ,  $p = 0.009$ ) (Table 2). When the two groups were compared in terms of CASP3 concentration values, there were statistically significant differences between mild and severe groups ( $\chi^2 = 4.730$ ,  $p = 0.030$ ) and between mild and critical groups ( $\chi^2 = 6.334$ ,  $p = 0.012$ ) (Table 2).

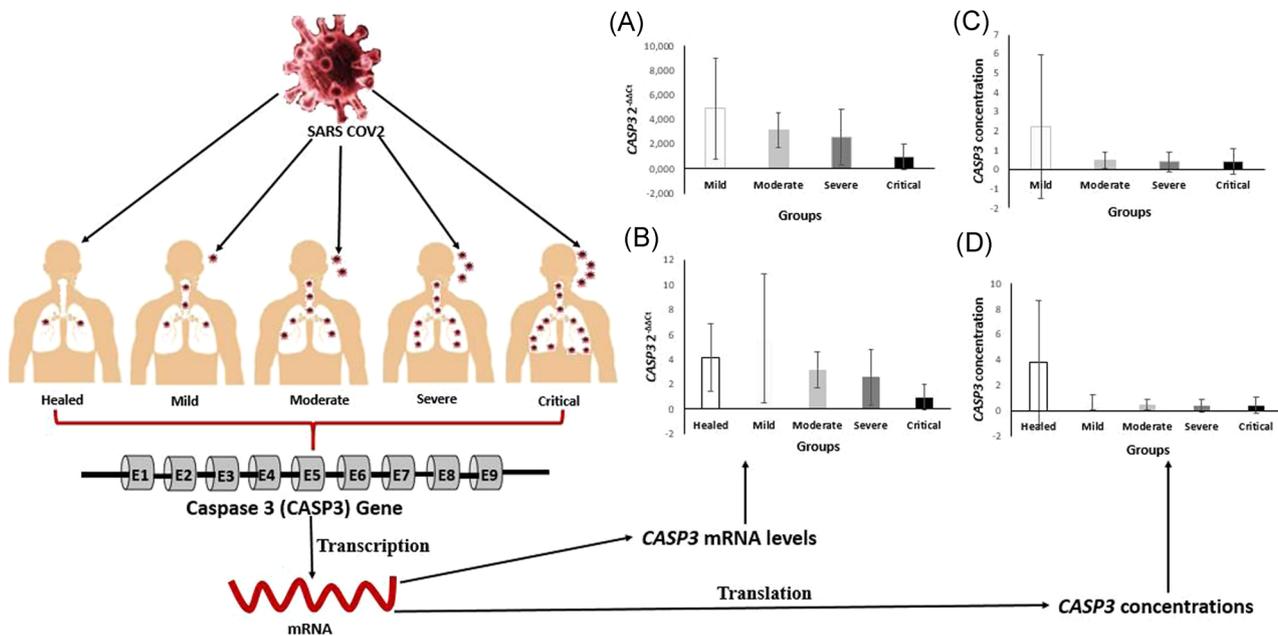
The Mean  $\pm$  SD Hemogram values of each group are given in the Table 1. When all groups were taken into consideration, statistically significant differences were detected among the groups for ferritin

**TABLE 1** The Hemogram values, CASP3  $2^{-\Delta\Delta C_t}$  and CASP3 concentration levels of each group

	Groups				$\chi^2/p$
	Mild (n = 16)	Moderate (n = 9)	Severe (n = 8)	Critical (n = 8)	
Age (years)	40.188 $\pm$ 15.272	58.222 $\pm$ 11.355	70.375 $\pm$ 12.247	71.875 $\pm$ 12.264	20.210/0.000*
HB	12.588 $\pm$ 1.767	12.756 $\pm$ 2.094	13.213 $\pm$ 2.389	12.813 $\pm$ 1.132	1.183/0.757
PLT	203.750 $\pm$ 54.176	281.667 $\pm$ 140.000	314.000 $\pm$ 102.271	230.375 $\pm$ 134.430	5.587/0.134
Lymphocyte	671.250 $\pm$ 166.342	652.222 $\pm$ 326.488	596.250 $\pm$ 226.838	573.750 $\pm$ 254.948	0.991/0.803
CRP	2.824 $\pm$ 2.707	7.076 $\pm$ 5.490	7.249 $\pm$ 9.464	7.933 $\pm$ 6.678	4.322/0.229
Ferritin	206.400 $\pm$ 136.862	549.111 $\pm$ 246.763	569.250 $\pm$ 420.320	1145.625 $\pm$ 502.592	13.459/0.004*
D-dimer	0.391 $\pm$ 0.185	0.762 $\pm$ 0.575	1.133 $\pm$ 1.249	6.153 $\pm$ 7.582	11.588/0.009*
LDH	315.125 $\pm$ 152.031	305.111 $\pm$ 92.669	349.250 $\pm$ 103.989	716.250 $\pm$ 235.635	16.421/0.001*
SpO <sub>2</sub>	95.563 $\pm$ 1.153	90.444 $\pm$ 3.046	82.125 $\pm$ 5.817	68.625 $\pm$ 7.873	35.260/0.000*
CASP3 $2^{-\Delta\Delta C_t}$	4.923 $\pm$ 4.098	3.153 $\pm$ 1.429	2.576 $\pm$ 2.244	0.955 $\pm$ 1.044	10.609/0.014*
CASP3 conc.	2.225 $\pm$ 3.747	0.467 $\pm$ 0.423	0.394 $\pm$ 0.509	0.415 $\pm$ 0.654	9.427/0.024*

Abbreviations: CASP3, Caspase 3; Conc, concentration; CRP, C-reactive protein; HB, hemoglobin; LDH, lactate dehydrogenase; PLT, platelet; SpO<sub>2</sub>, oxygen saturation level in the blood.

\*Statistically significant.



**FIGURE 1** Experimental demonstration of SARS-CoV-2 infection. The patients were divided into four main groups such as mild (patients with mild illness and healed from the disease, and patients with mild disease), moderate, severe, and critical. Both  $CASP3\ 2^{-\Delta\Delta C_t}$  levels and  $CASP3$  concentration levels of patients were detected. SARS-CoV-2 infection caused cellular damage and a response occurs in the nucleus of cells.  $CASP3$  gene is transcribed  $CASP3$  mRNA and  $CASP3$  proteins are translated from mature  $CASP3$  mRNA. Statistically significant differences were detected among all groups for both  $CASP3\ 2^{-\Delta\Delta C_t}$  levels (A and B) and  $CASP3$  concentration levels of patients (C and D).  $CASP3$ , Caspase-3; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2

( $\chi^2 = 13.459$ ,  $p = 0.004$ ), for D-dimer ( $\chi^2 = 11.588$ ,  $p = 0.009$ ), for LDH ( $\chi^2 = 16.421$ ,  $p = 0.001$ ) and for  $SpO_2$  ( $\chi^2 = 35.260$ ,  $p < 0.001$ ), respectively (Table 1). To understand these differences' causes from which groups, double comparison of the groups was carried out. Statistically significant differences between mild ( $206.400 \pm 136.862$ ) and moderate ( $549.111 \pm 246.763$ ) ( $\chi^2 = 5.444$ ,  $p = 0.020$ ), between mild ( $206.400 \pm 136.862$ ) and critical ( $1145.625 \pm 502.592$ ) ( $\chi^2 = 8.571$ ,  $p = 0.003$ ), between moderate ( $549.111 \pm 246.763$ ) and critical ( $1145.625 \pm 502.592$ ) ( $\chi^2 = 6.510$ ,  $p = 0.011$ ), between severe ( $569.250 \pm 420.320$ ) and critical ( $1145.625 \pm 502.592$ ) ( $\chi^2 = 3.982$ ,  $p = 0.046$ ) were detected for ferritin (Table 2). The ferritin values of the patients included in the study were critical ( $1145.625 \pm 502.592$ ), severe ( $569.250 \pm 420.320$ ), moderate ( $549.111 \pm 246.763$ ), and mild ( $206.400 \pm 136.862$ ) from the highest to the lowest, respectively (Table 1).

When the two groups were compared in terms of D-dimer, statistically significant differences between mild ( $0.391 \pm 0.185$ ) and severe ( $1.133 \pm 1.249$ ) ( $\chi^2 = 6.904$ ,  $p = 0.009$ ), between mild ( $0.391 \pm 0.185$ ) and critical ( $6.153 \pm 7.582$ ) ( $\chi^2 = 9.303$ ,  $p = 0.002$ ), between moderate ( $0.762 \pm 0.575$ ) and critical ( $6.153 \pm 7.582$ ) ( $\chi^2 = 3.717$ ,  $p = 0.054$ ) were detected (Table 2). The D-dimer values of the patients included in the study were critical ( $6.153 \pm 7.582$ ), severe ( $1.133 \pm 1.249$ ), moderate ( $0.762 \pm 0.575$ ) and mild ( $0.391 \pm 0.185$ ) from the highest to the lowest, respectively (Table 1).

Also, statistically significant differences between mild ( $315.125 \pm 152.031$ ) and critical ( $716.250 \pm 235.635$ ) ( $\chi^2 = 9.276$ ,  $p = 0.002$ ), between moderate ( $305.111 \pm 92.669$ ) and critical ( $716.250 \pm$

$235.635$ ) ( $\chi^2 = 12.000$ ,  $p = 0.001$ ), between severe ( $349.250 \pm 103.989$ ) and critical ( $716.250 \pm 235.635$ ) ( $\chi^2 = 9.926$ ,  $p = 0.002$ ) were detected for LDH (Table 2). The LDH values of the patients included in the study were critical ( $716.250 \pm 235.635$ ), severe ( $349.250 \pm 103.989$ ), mild ( $315.125 \pm 152.031$ ), and moderate ( $305.111 \pm 92.669$ ) from the highest to the lowest, respectively (Table 1).

Additionally, statistically significant differences between mild ( $95.563 \pm 1.153$ ) and moderate ( $90.444 \pm 3.046$ ) ( $\chi^2 = 14.821$ ,  $p < 0.001$ ), between mild ( $95.563 \pm 1.153$ ) and severe ( $82.125 \pm 5.817$ ) ( $\chi^2 = 15.835$ ,  $p < 0.001$ ), between mild ( $95.563 \pm 1.153$ ) and critical ( $68.625 \pm 7.873$ ) ( $\chi^2 = 15.842$ ,  $p < 0.001$ ), between moderate ( $90.444 \pm 3.046$ ) and critical ( $68.625 \pm 7.873$ ) ( $\chi^2 = 12.134$ ,  $p < 0.001$ ), between severe ( $82.125 \pm 5.817$ ) and critical ( $68.625 \pm 7.873$ ) ( $\chi^2 = 8.802$ ,  $p = 0.003$ ) were detected for  $SpO_2$  (Table 2). The  $SpO_2$  values of the patients included in the study were critical ( $68.625 \pm 7.873$ ), severe ( $82.125 \pm 5.817$ ), moderate ( $90.444 \pm 3.046$ ), and mild ( $95.563 \pm 1.153$ ) from the lowest to the highest, respectively (Table 1).

When we divided Group 1 into two subgroup such as patients with mild illness and healed from the disease, and patients with mild disease, statistically significant differences were detected among all groups for age ( $\chi^2 = 24.526$ ;  $p < 0.001$ ),  $CASP3\ 2^{-\Delta\Delta C_t}$  ( $\chi^2 = 10.613$ ;  $p = 0.031$ ) and  $CASP3$  concentration ( $\chi^2 = 12.401$ ;  $p = 0.015$ ) (Table 3). According to this grouping, the  $CASP3\ 2^{-\Delta\Delta C_t}$  values of the patients included in the study were critical ( $0.955 \pm 1.044$ ), severe ( $2.576 \pm 2.244$ ), moderate ( $3.153 \pm 1.429$ ), healed ( $4.141 \pm 2.745$ ), and mild ( $5.704 \pm 5.203$ ) from the lowest to the highest, respectively (Table 3) (Figure 1B). The  $CASP3$  concentration values of the patients

**TABLE 2** Binary comparison of the groups for CASP3 2<sup>-ΔΔC<sub>t</sub></sup>, CASP3 concentration, and blood parameters

Grps	Mild		Moderate		Severe		Critical		
	χ <sup>2</sup>	p							
CASP3 2 <sup>-ΔΔC<sub>t</sub></sup>									
Mild	-	-	0.821	0.365	1.577	0.209	8.825	0.003*	
Moderate	0.821	0.365	-	-	0.037	0.847	6.750	0.009*	
Severe	1.577	0.209	0.037	0.847	-	-	2.824	0.093	
Critical	8.825	0.003*	6.750	0.009*	2.824	0.093	-	-	
CASP3 Conc									
Mild	-	-	3.492	0.062	4.730	0.030*	6.334	0.012*	
Moderate	3.492	0.062	-	-	0.280	0.596	0.949	0.330	
Severe	4.730	0.030*	0.280	0.596	-	-	0.557	0.456	
Critical	6.334	0.012*	0.949	0.330	0.557	0.456	-	-	
Ferritin									
Mild	-	-	5.444	0.020*	3.086	0.079	8.571	0.003*	
Moderate	5.444	0.020*	-	-	0.083	0.773	6.510	0.011*	
Severe	3.086	0.079	0.083	0.773	-	-	3.982	0.046*	
Critical	8.571	0.003*	6.510	0.011*	3.982	0.046*	-	-	
D-dimer									
Mild	-	-	1.129	0.288	6.904	0.009*	9.303	0.002*	
Moderate	1.129	0.288	-	-	0.455	0.500	3.717	0.054*	
Severe	6.904	0.009*	0.455	0.500	-	-	1.867	0.172	
Critical	9.303	0.002*	3.717	0.054*	1.867	0.172	-	-	
LDH									
Mild	-	-	0.333	0.564	0.397	0.529	9.276	0.002*	
Moderate	0.333	0.564	-	-	0.750	0.386	12.000	0.001*	
Severe	0.397	0.529	0.750	0.386	-	-	9.926	0.002*	
Critical	9.276	0.002*	12.000	0.001*	9.926	0.002*	-	-	
SpO <sub>2</sub>									
Mild	-	-	14.821	<0001*	15.835	<0001*	15.842	<0001*	
Moderate	14.821	<0001*	-	-	10.280	0.001	12.134	<0001*	
Severe	15.835	<0001*	10.280	0.001	-	-	8.802	0.003*	
Critical	15.842	<0001*	12.134	0.000*	8.802	0.003*	-	-	

Abbreviations: CASP3, Caspase 3; Conc, concentration; LDH, lactate dehydrogenase; SpO<sub>2</sub>, Oxygen saturation level in the blood.

\*Statistically significant.

**TABLE 3** The age, CASP3 2<sup>-ΔΔC<sub>t</sub></sup>, and CASP3 concentration levels of each group when Group 1 divided into two subgroup such as patients with mild illness and healed from the disease, and patients with mild disease

	Groups					χ <sup>2</sup> /p
	Healed (n = 8)	Mild (n = 8)	Moderate (n = 9)	Severe (n = 8)	Critical (n = 8)	
Age (years)	30.375 ± 2.875	50.000 ± 16.475	58.222 ± 11.355	70.375 ± 12.247	71.875 ± 12.264	24.526/0.000*
CASP3 2 <sup>-ΔΔC<sub>t</sub></sup>	4.141 ± 2.745	5.704 ± 5.203	3.153 ± 1.429	2.576 ± 2.244	0.955 ± 1.044	10.613/0.031*
CASP3conc.	3.779 ± 4.920	0.671 ± 0.597	0.467 ± 0.423	0.394 ± 0.509	0.415 ± 0.654	12.401/0.015*

Abbreviations: CASP3, caspase 3; conc, concentration.

\*Statistically significant

included in the study were severe ( $0.394 \pm 0.509$ ), critical ( $0.415 \pm 0.654$ ), moderate ( $0.467 \pm 0.423$ ), mild ( $0.671 \pm 0.597$ ), and healed ( $3.779 \pm 4.920$ ) from the lowest to the highest, respectively (Table 3) (Figure 1D).

Although the  $CASP3\ 2^{-\Delta\Delta C_t}$  values of the patients with mild group is approximately 1.4 times higher than the healed group, the  $CASP3$  concentration values of the patients with healed group is approximately 5.6 times higher in the healed group than in the mild group.

Additionally, statistically significant differences were detected among all groups for sex ( $\chi^2 = 9.092$ ,  $p = 0.028$ ), additional disease ( $\chi^2 = 17.338$ ,  $p = 0.001$ ), HT ( $\chi^2 = 11.167$ ,  $p = 0.011$ ), and lungs ( $\chi^2 = 10.871$ ,  $p = 0.012$ ) (Table 4).

When the both  $CASP3\ 2^{-\Delta\Delta C_t}$  and  $CASP3$  concentration levels of the related genes and blood parameters to be considered, the relationships between  $CASP3\ 2^{-\Delta\Delta C_t}$  levels and HB ( $p = 0.023$ ), between  $CASP3\ 2^{-\Delta\Delta C_t}$  levels and CRP ( $p = 0.001$ ), between  $CASP3\ 2^{-\Delta\Delta C_t}$  levels and ferritin ( $p = 0.003$ ), between  $CASP3\ 2^{-\Delta\Delta C_t}$  levels and LDH ( $p = 0.001$ ), and between  $CASP3\ 2^{-\Delta\Delta C_t}$  levels and  $SpO_2$  ( $p = 0.006$ ) were statistically significant (Table 5 and Figure 2). Additionally, the relationship between  $CASP3$  concentration levels and  $SpO_2$  was statistically significant ( $p < 0.046$ ) (Table 5 and Figure 2), too.

Experimental demonstration of SARS-CoV-2 infection is given in Figure 1. The patients were divided into four main groups such as mild (patients with mild illness and healed from the disease, and patients with mild disease), moderate, severe, and critical. Both  $CASP3\ 2^{-\Delta\Delta C_t}$  levels and  $CASP3$  concentration levels of patients were detected. SARS-CoV-2 infection caused cellular damage and a response occurs in the nucleus of cells. So  $CASP3$  gene is transcribed  $CASP3$  mRNA and  $CASP3$  proteins are translated from mature  $CASP3$  mRNA. Statistically significant differences were detected among all groups for both  $CASP3\ 2^{-\Delta\Delta C_t}$  levels (a and b) and  $CASP3$  concentration levels of patients (c and d) (Figure 1).

## 4 | DISCUSSION

In this study,  $CASP3\ 2^{-\Delta\Delta C_t}$  levels increased as poor prognostic factors (LDH, ferritin, D-dimer, and HB) for SARS-CoV-2 infection increased. Also, the relationship between serum  $CASP3$  concentration levels and  $SpO_2$  was statistically significant.

Statistically significant differences were detected among all groups for patient ages. The disease severity of the individuals included in the study was critical, severe, moderate, and mild, ranging from the oldest to the youngest. Therefore, the disease progresses from being more severe in the elderly compared with the young. This difference might be due to the increased risk of cellular ageing and the decrease in metabolic rate, increasing the risk of other diseases.

Zhang et al. showed that EV71-induced T-lymphocyte apoptosis has a crucial function in hand, foot, and mouth disease. They indicated that EV71 infection significantly induced apoptosis in human T-lymphocytes, increased the pro-apoptosis Bcl-2-associated X (Bax) expression, and the cleaved  $CASP3$  conversely decreased the anti-apoptosis B-cell lymphoma protein 2 (Bcl2) expression.<sup>7</sup> Chen et al. reported, and we verified that viral 2Apro could activate cellular  $CASP3$ . The inhibition of  $CASP3$  activation could lead to the partial restoration of interferon-alpha receptor 1 (IFNAR1) in cells transfected with 2A or infected with EV71.<sup>8</sup> Another study showed that EV71 induced apoptosis in a  $CASP3$ -dependent pathway.<sup>9</sup> Also, Guo et al. reported that acute kidney injury is a common complication of caecal ligation and puncture-induced sepsis. Thymoquinone protected against kidney damage and inhibited increases in serum creatinin and blood urea nitrogen levels, high levels of NLR-family pyrin domain-containing protein 3, caspase-1,  $CASP3$ , caspase-8, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-1 $\beta$  (IL-1 $\beta$ ), and IL-6 in septic BALB/c mice.<sup>15</sup> Moehler et al. reported that hepatoma cell death correlated with parvovirus H1 DNA replication, morphological features of apoptosis, and nonstructural protein expression.

**TABLE 4** The sex and additional diseases of each group

	Groups				$\chi^2/p$
	Mild (n = 16)	Moderate (n = 9)	Severe (n = 8)	Critical (n = 8)	
Sex (M/F)	4(25%)/12(75%)	5(55.6%)/4(44.4%)	5(62.5%)/3(37.5%)	7(87.5%)/1(12.5%)	9.092/0.028*
AD (Y/N)	3(18.8%)/13(81.2%)	7(77.8%)/2(22.2%)	7(87.5%)/1(12.5%)	7(87.5%)/1(12.5%)	17.338/0.001*
DM (Y/N)	2(12.5%)/14(87.5%)	5(55.6%)/4(44.4%)	2(25%)/6(75%)	2(25%)/6(75%)	5.484/0.140
HT (Y/N)	2(12.5%)/14(87.5%)	5(55.6%)/4(44.4%)	6(75%)/2(25%)	5(62.5%)/3(37.5%)	11.167/0.011*
CVA (Y/N)	0(0%)/16(100%)	1(11.1%)/8(88.9%)	0(0%)/8(100%)	2(25%)/6(75%)	5.774/0.123
CRD (Y/N)	0(0%)/16(100%)	1(11.1%)/8(88.9%)	4(50%)/4(50%)	1(12.5%)/7(87.5%)	10.871/0.012*
CRF (Y/N)	0(0%)/16(100%)	1(11.1%)/8(88.9%)	0(0%)/8(100%)	0(0%)/8(100%)	2.390/0.303
Malig (Y/N)	0(0%)/16(100%)	1(11.1%)/8(88.9%)	0(0%)/8(100%)	2(25%)/6(75%)	12.228/0.201
CVD (Y/N)	3(18.8%)/13(81.2%)	3(33.3%)/6(66.7%)	2(25%)/6(75%)	1(12.5%)/7(87.5%)	1.237/0.744

Abbreviations: AD, additional Disease; CVA: Cerebrovascular accident; CRF, chronic renal failure; CVD, cardiovascular disease; CRD, chronic respiratory disease; DM, diabetes mellitus; HT, hypertension; Malig, Malignancy; M, male; N, no; f, female; Y, yes.

\*Statistically significant.

**TABLE 5** Model summary and parameter estimates for between both  $CASP3\ 2^{-\Delta\Delta C_t}$  and  $CASP3$  concentration levels and hemogram parameters

Variable	Equation	Model summary					Parameter estimates			
		$R^2$	F	df1	df2	sig	Constant	b1	b2	b3
$CASP3\ 2^{-\Delta\Delta C_t}$ and HB	Linear	0.157	5.761	1	31	0.023	12.162	-0.706		
	Log	0.155	5.704	1	31	0.023	24.263	-8.327		
	Cubic	0.157	2.796	2	30	0.077	13.477	-0.870	0.000	0.000
$CASP3\ 2^{-\Delta\Delta C_t}$ and CRP	Linear	0.102	3.526	1	31	0.070	4.107	-0.160		
	Log	0.305	13.579	1	31	0.001	4.713	-1.337		
	Cubic	0.187	2.219	3	29	0.107	5.598	-1.050	0.090	-0.002
$CASP3\ 2^{-\Delta\Delta C_t}$ and Ferritin	Linear	0.276	10.656	1	28	0.003	3.738	-0.002		
	Log	0.199	6.973	1	28	0.013	8.015	-0.909		
	Cubic	0.291	3.560	3	26	0.028	3.630	-0.001	-2.567	1.081
$CASP3\ 2^{-\Delta\Delta C_t}$ and LDH	Linear	0.170	6.357	1	31	0.017	5.585	-0.006		
	Log	0.300	13.272	1	31	0.001	25.178	-3.731		
	Cubic	0.437	7.508	3	29	0.001	19.222	-0.085	0.000	-5.124
$CASP3\ 2^{-\Delta\Delta C_t}$ and SpO <sub>2</sub>	Linear	0.179	8.522	1	39	0.006	-7.163	0.121		
	Log	0.168	7.896	1	39	0.008	-37.893	9.254		
	Cubic	0.211	5.082	2	38	0.011	13.554	-0.277	0.000	2.027
$CASP3$ Conc. and SpO <sub>2</sub>	Linear	0.057	2.346	1	39	0.134	-3.500	0.053		
	Log	0.048	1.972	1	39	0.168	-16.191	3.890		
	Cubic	0.150	3.343	2	38	0.046	12.069	0.000	-0.006	5.339
$CASP3\ 2^{-\Delta\Delta C_t}$ and Lymph.	Linear	0.005	0.141	1	31	0.710	2.538	0.001		
	Logarithmic	0.006	0.178	1	31	0.676	-0.765	0.607		
	Cubic	0.006	0.058	3	29	0.982	1.124	0.007	-7.726	2.768
$CASP3$ Conc. and Lymph.	Linear	0.018	0.573	1	31	0.455	0.669	0.000		
	Logarithmic	0.028	0.880	1	31	0.355	1.872	-0.218		
	Cubic	0.182	2.144	3	29	0.116	-0.909	0.009	-1.398	6.346

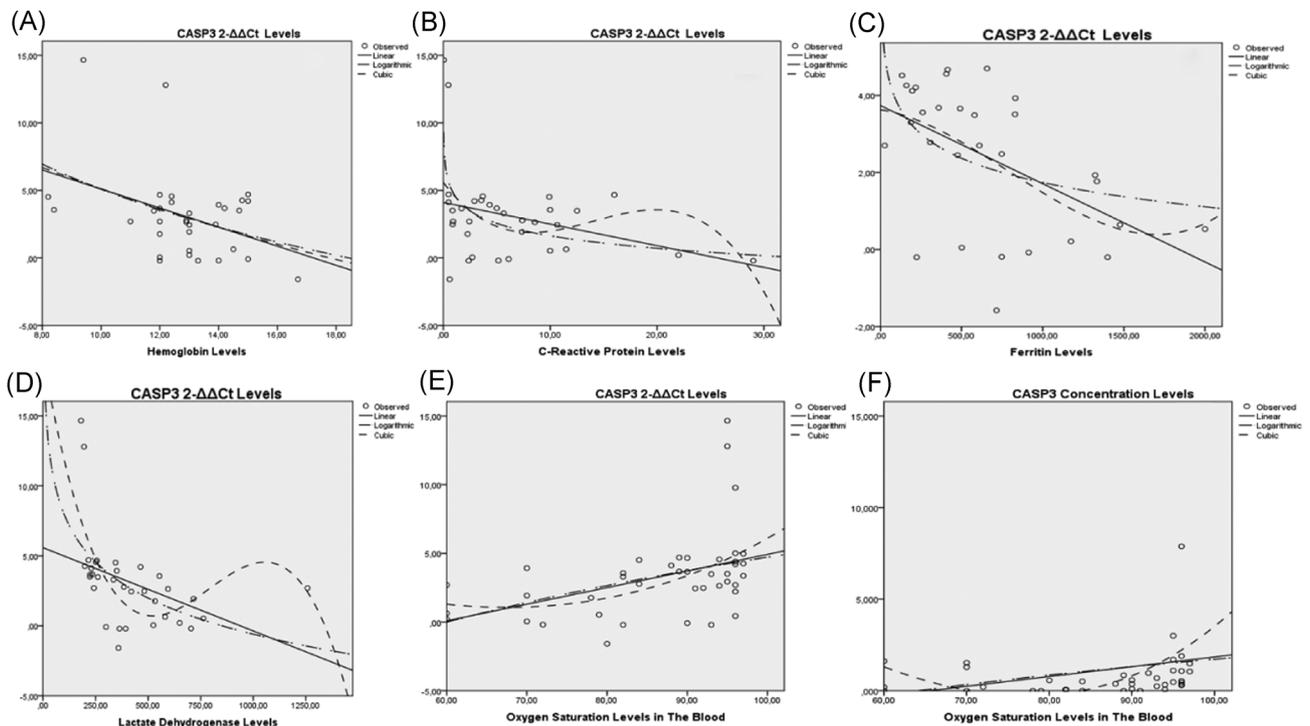
Abbreviations:  $CASP3$ , caspase 3; conc, concentration; HB, hemoglobin; PLT, Platelet; CRP, C-reactive protein; LDH, lactate dehydrogenase; Lymph, lymphocytes; SpO<sub>2</sub>, oxygen saturation level in the blood.

Parvovirus H1 induced significant apoptosis. Apoptosis was partially inhibited by DEVD-CHO, a  $CASP3$  inhibitor.<sup>10</sup> The expression levels of  $CASP3$  were increased and activated in the human cytomegalovirus-infected cells, in addition to downregulating *bel-2* mRNA expression and upregulating *fas* mRNA expression.<sup>11</sup> In addition, Allhethel et al. reported that, while the expression levels of signal transducer and activator of transcriptio-1 and Interferon regulatory factor -1 were significantly downregulated, the expression of  $CASP3$  was significantly upregulated in peripheral blood mononuclear cells of patients infected with the hepatitis C virus.<sup>12</sup> Also,  $CASP3$ , 8 and 9 activities and the interferon promoter terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling were strongly induced in early infection in H5N1 Cambodia PA-infected cells that protect uninfected cells from viral infection.<sup>13</sup> In addition, the Caprine parainfluenza virus type 3, an emerging respiratory

pathogen, triggers apoptosis by targeting  $CASP3$ , an effector of apoptosis.<sup>14</sup>

In our study, we found a significant relationship between  $CASP3\ 2^{-\Delta\Delta C_t}$  level and disease severity. Statistically significant differences were detected among all groups for  $CASP3\ 2^{-\Delta\Delta C_t}$  ( $p = 0.014$ ) and  $CASP3$  concentration ( $p = 0.024$ ). When the two groups were compared regarding  $CASP3\ 2^{-\Delta\Delta C_t}$  values, there were statistically significant differences between mild and critical groups ( $p = 0.003$ ) and between the moderate and critical groups ( $p = 0.009$ ). Also, statistically significant differences between the mild and severe groups ( $p = 0.030$ ) and between the mild and critical groups were detected for  $CASP3$  concentration values ( $p = 0.012$ ). Perhaps,  $CASP3$  levels can provide information about disease severity?

Although the  $CASP3\ 2^{-\Delta\Delta C_t}$  values of patients in the mild group is approximately 1.4 times higher than the healed group, the  $CASP3$



**FIGURE 2** Relationship among both CASP3  $2^{-\Delta\Delta C_t}$  levels and CASP3 concentration levels of patients and blood parameters. Statistically significant relation were detected between CASP3  $2^{-\Delta\Delta C_t}$  levels and hemoglobin (A), between CASP3  $2^{-\Delta\Delta C_t}$  levels and CRP (B), between CASP3  $2^{-\Delta\Delta C_t}$  levels and ferritin (C), between CASP3  $2^{-\Delta\Delta C_t}$  levels and LDH (D) and between CASP3  $2^{-\Delta\Delta C_t}$  levels and SpO<sub>2</sub> (E). Additionally, the relationship between CASP3 concentration levels and SpO<sub>2</sub> was statistically significant ( $p < 0.046$ ) (F), too. CASP3, Caspase-3; CRP, C-reactive protein; LDH, lactate dehydrogenase

concentration values of patients in the healed group are approximately 5.6 times higher in the healed group than in the mild group. Therefore, CASP3 plays a vital role in protecting against SARS-CoV-2 infection. CASP3 gene expression starts to increase when the virus is encountered. Disease severity decreases in contrast to the increasing expression capacity and amount of CASP3. CASP3 gene product is found in high amounts in healed individuals. The levels of CASP3 gene expression and/or its products may vary depending on the degree of cellular damage caused by SARS-CoV-2 infection. This process may continue until the balance of the cells is restored when homeostasis is achieved. To obtain more certain knowledge about the current topic additional studies about the activity level of the CASP3 are needed.

CASP3 has an important function to maintain the homeostasis of living cells. CASP3 could be used as an indicator to evaluate the degree of the cell damage caused by the SARS-CoV-2 infection. Also, CASP3 could be used as a biomarker for selecting the most reliable treatment strategy and then evaluating the success rate of the treatment strategy. All living cells must protect themselves from internal and external dangerous agents such as SARS-CoV-2 infection. CASP3 is activated against injury caused by SARS-CoV-2 infection as a protective mechanism or triggers the expression of other genes and their products. What role does CASP3 play in regulating gene expression and signaling transduction pathways against injury caused by SARS-CoV-2 infection? Can CASP3 be used as a therapeutic agent to prevent injury caused by

SARS-CoV-2 infection? This approach might lead to developing new therapeutic options against injury caused by SARS-CoV-2 infection in the near future. Can these processes protect uninfected cells from a viral infection? Additional studies about the current topic should be conducted to understand this situation more clearly.

Studies reported that as HB<sup>16,17</sup> and lymphocyte<sup>18</sup> levels decrease, LDH,<sup>19,20</sup> D-dimer,<sup>21-23</sup> ferritin<sup>24,25</sup> levels increase and the severity of disease increases.

When all groups were considered, statistically significant differences were detected among the groups for ferritin ( $p = 0.004$ ), D-dimer ( $p = 0.009$ ), LDH ( $p = 0.001$ ), and SpO<sub>2</sub> ( $p < 0.001$ ).

Statistically significant reverse relations were detected between CASP3  $2^{-\Delta\Delta C_t}$  levels and HB, between CASP3  $2^{-\Delta\Delta C_t}$  levels and CRP, between CASP3  $2^{-\Delta\Delta C_t}$  levels and ferritin, and between CASP3  $2^{-\Delta\Delta C_t}$  levels and LDH. Conversely, statistically significant relations between CASP3  $2^{-\Delta\Delta C_t}$  levels and SpO<sub>2</sub> and between CASP3 concentration levels and SpO<sub>2</sub> were detected.

## 5 | CONCLUSION

The CASP3 gene and/or its products have an important function to prevent injury caused by SARS-CoV-2 infection. They play crucial roles in maintaining cellular homeostasis and viability. Perhaps CASP3

levels can provide information about the severity of the COVID-19 disease. Further studies are needed on this subject.

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## CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

## AUTHOR CONTRIBUTIONS

**Data collection:** Pinar Yildiz Gulhan, Recep Eroz, Ozlem Ataoglu, Nevin Ince, Fatih Davran, Cihadiye Elif Öztürk, Zerrin Gamsızkan. **Study design:** Pinar Yildiz Gulhan, Recep Eroz. **Analysis of data:** Pinar Yildiz Gulhan, Recep Eroz, Ozlem Ataoglu, Cihadiye Elif Öztürk, Oner Abidin Balbay. **Manuscript preparation:** Pinar Yildiz Gulhan, Recep Eroz, Ozlem Ataoglu, Nevin Ince, Fatih Davran. **Review of manuscript:** Pinar Yildiz Gulhan, Recep Eroz, Ozlem Ataoglu, Nevin Ince, Fatih Davran, Cihadiye Elif Öztürk, Oner Abidin Balbay, Zerrin Gamsızkan

## DATA AVAILABILITY STATEMENT

Due to the nature of this study, participants of this study did not agree for their data to be shared publicly, so supporting data is not available.

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