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Induction of Caspase-7 Serum and P^{53} Gene Expression in Severe Omicron Variant SARS-CoV-2 Infection

Rasha J. Khalaf, Hula Y. Fadhil *

Department of biology, college of science, university of Baghdad, Baghdad, AL-Jadiria, Iraq

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Abstract

The COVID-19 pandemic has caused significant global health issues, with the Omicron variant emerging in South Africa in November 2021. Apoptosis is a natural antiviral defense; however, in SARS-CoV-2 infection, it may paradoxically enhance viral replication, worsening the severity instead of eradicating it. This study aims to investigate viral infection by analyzing serum levels of caspase-7 and gene expression of P^{53} during the severe and late stages of Omicron infection, as well as their correlation with severity, inflammatory biomarkers, and blood cell counts. A case-control study involved 124 participants aged 25-69, with 74 being SARS-CoV-2 patients and 50 healthy controls. The study found that patients had significantly lower median levels of caspase-7 and P^{53} than healthy controls. Most patients were considered low producers of caspase-7 and P^{53} . A caspase-7 level greater than 3169 pg/ml (area under the curve=0.790; $p < 0.0001$) in receiver operating characteristic curve (ROC) analysis; therefore, it is considered a good marker to predict the outcome of COVID-19 patients. The caspase-7 levels showed a significant relationship with biomarkers (ferritin, D-dimer, and blood cells). Our findings with the Omicron variant revealed low levels of caspase-7 and P^{53} at early infection stages and in older patients with a low Ct value (indicating a high viral load). Thus, the P^{53} expression induces caspase-7 production, affecting the viral load outcome and control. Further research on the relationship between the severity of Omicron SARS-CoV-2 infections and the activity level of apoptotic caspases is necessary to fully comprehend the hidden potential and characteristics of the current subject of study.

Keywords: SARS-CoV-2, Omicron variant, Tumor suppressor protein P^{53} , Caspase-7, Biomarkers, Real-time PCR

تحفيز مستوى كاسباس-7 في المصل في عدوى فيروس كورونا المستجد 2 (SARS-CoV-2)
لمتغير اوميكرون في الحالات الشديدة والمتأخرة

رشا جاسم خلف, حلا يونس فاضل *

قسم علوم الحياة، كلية العلوم، جامعة بغداد، بغداد، العراق

الخلاصة

جائحة كوفيد-19 تسببت في حدوث مشكلات صحية عالمية كبيرة، مع ظهور متحور أوميكرون في

*Email: hula.younis@sc.uobaghdad.edu.iq



جنوب إفريقيا في نوفمبر 2021. قد يُعزز موت الخلايا المبرمج، وهو دفاع قوي مضاد للفيروسات، عدوى فيروس كورونا المستجد SARS-CoV-2 أحياناً، مما قد يُعاقم شدتها بدلاً من القضاء عليها. تهدف هذه الدراسة إلى التخفيف من حدة العدوى الفيروسية من خلال دراسة مستويات كاسباس-7 في المصل والتعبير الجيني لبروتين P53 خلال المراحل الحادة والمتأخرة من عدوى أوميكرون، بالإضافة إلى ارتباطها بشدة العدوى، والعلامات الحيوية الالتهابية، وعدد خلايا الدم. شملت الدراسة مقارنة حالة المرضى مع الأصحاء لـ 124 مشاركاً تتراوح أعمارهم بين 25 و69 عاماً، منهم 74 مصاباً بفيروس كورونا المستجد SARS-CoV-2 و50 من الأشخاص الأصحاء. وجدت الدراسة أن متوسط مستويات كاسباس-7 وP53 لدى المرضى كان أقل بكثير من متوسط مستويات الكاسباس-7 وP53 لدى الأصحاء. اعتُبر معظم المرضى منتجين منخفضين لكاسباس-7 وP53. أظهر تحليل منحنى المميز للتشغيل المتلقي (ROC) أن مستوى كاسباس-7 كان أعلى من 3169 بيكو غرام/مل (المساحة تحت المنحنى = 0.790؛ $p < 0.0001$)؛ لذلك، يُعتبر مؤشراً جيداً للتنبؤ بنتائج مرضى كوفيد-19. أظهرت مستويات كاسباس-7 علاقة وثيقة بالمؤشرات الحيوية (الفيبريتين، وثنائي-دي، وخلايا الدم). وكشفت نتائجنا المتعلقة بمتحور أوميكرون عن انخفاض مستويات كاسباس-7 وP53 في المراحل المبكرة من العدوى، ولدى المرضى الأكبر سناً الذين لديهم قيمة Ct منخفضة (مما يشير إلى ارتفاع الحمل الفيروسي). وبالتالي، يُحفّز التعبير الجيني لجين P53 إنتاج كاسباس-7، مما يؤثر على نتائج الحمل الفيروسي والتحكم فيه. ومن الضروري إجراء المزيد من البحوث حول العلاقة بين شدة عدوى فيروس أوميكرون SARS-CoV-2 ومستوى نشاط كاسباسات موت الخلايا المبرمج لفهم الإمكانيات والخصائص الخفية لموضوع الدراسة الحالي بشكل كامل.

1 - Introduction

The World Health Organization (WHO) declared the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) a global pandemic on March 11, 2020, after it was identified in China at the end of 2019 [1]. The SARS-CoV-2 pandemic, which causes COVID-19, has given rise to a novel variant of concern (VOC) called Omicron. Omicron has spread rapidly in every country where it has been introduced, following its discovery in South Africa in November 2021 [2]. These new VOCs have the potential to become more transmissible due to the impact on the efficacy of monoclonal or vaccine-induced neutralizing antibodies, in addition to several other causes, such as the decrease of community defenses, the drift of antigens, as well as enhanced infectious capacity and some resistance to prior immunization [3]. Furthermore, to evade host immune surveillance, Omicron variants may generally employ three primary immune evasion strategies: impairment of humoral immunity, delays in cellular immunity, and alterations in innate immunity, including changes in apoptosis-related proteins, tumor suppressor protein P⁵³, and cytokine storms [4]. This suggests that Omicron variants greatly facilitate immune evasion.

The molecular mechanisms that control and carry out virus-induced apoptosis are unclear. However numerous cells respond to viral infection through apoptosis, mediated by caspases — particularly the executioner caspases (caspase-3 and caspase-7), which are activated by initiator caspases (e.g., caspase-9) and that cause cleaving and breaking down hundreds of distinct cellular substrates that are essential for cell survival, this promotes programmed cell death, as well as reduces the amount of virus progeny released. Apoptotic cells undergo a unique and well-organized set of morphological alterations as a direct result of this intricate process, ultimately leading to their systematic disintegration and death [5]. Moreover, viruses need living cells to copy their genetic material and produce offspring. Additionally, it increases their long-term viability within the host cell and their ability to propagate to neighboring host cells. As a result, viruses have developed numerous ways to alter the apoptosis of host cells by interfering with the functions of caspases, which primarily regulate apoptosis and inflammation in both immunological and non-immune cells during homeostasis and viral infection. Also, other unique mechanisms can give the host cell or virus a selective

survival advantage: (i) by protecting the cell against death too soon during lytic virus infection, which may lead to the release of more offspring virions, and (ii) by promoting the preservation and changing of host cells throughout latent infections [6].

Since apoptosis facilitates the virus's escape from the infected host cell and enables it to spread to other cells, it may be advantageous to the virus. As a result, certain viruses can trigger apoptosis, after which they exploit the remaining host cells to spread their virus [7]. However, the programmed death of cells is increasingly seen as a double-edged sword in the context of viral infection. On the one hand, it represents the host's protective immune response against viral infection by directly preventing the spread of viruses through the stimulation of apoptotic caspases to eliminate infectious viruses and maintain cellular balance [8]. But, on the other hand, uncontrolled apoptosis can lead to a cytokine storm, tissue degradation, viral dissemination, dysfunctional immune responses, or even host death, especially in the late or severe stages of SARS-CoV-2 infection [9]. Furthermore, coronaviruses can control apoptosis and autophagy by either triggering or preventing them as needed to increase their survival and proliferation [10]. Due to the uniqueness and significance of apoptosis, a complex regulatory system network exists, and the p^{53} serves as one of its key links. The p^{53} is an essential protein that manages several basic cellular signaling pathways, including the cellular cycle, DNA damage repair, apoptosis, and the cellular stress response [11]. Other research has demonstrated that p^{53} may immediately induce mitochondrial dysfunction after viral infection, resulting in excessive reactive oxygen species (ROS) levels and triggering cell death [12]. Currently, there is insufficient information to understand the relationship between caspase-7(CASP7) and the severity of SARS-CoV-2 infection. To assess the involvement of CASP7 in the severity progression of SARS-CoV-2 in patients with critical, chronic, and severe sickness, a preliminary search was conducted, aimed to investigate viral infection by analyzing serum levels of CASP7 and gene expression of P^{53} during the severe stage of Omicron infection, as well as their correlation with severity, viral load, inflammatory biomarkers, and blood cell counts.

2-Materials and Methods

2.1 Study population

A case-control study comprised 124 Iraqi male and female participants aged between 25 and 69. Fifty healthy controls (had negative result of reverse transcriptase real-time PCR (rRT-PCR) and did not have autoimmune illnesses) and 74 patients with SARS-CoV2 illness who had upper and/or lower respiratory tract symptoms and who had previously been diagnosed positive of rRT-PCR by health ministry hospitals such as Al-Imamein Kadhimain Medical City, Yarmouk Teaching Hospital, as well as from Alshifaa Specialist Center. The experiments were conducted between February 2024 and November 2024 for the patient enrollment period. The cases were categorized as mild to moderate (61) and severe (13), according to the consulting doctor, diagnosis depends on clinical symptoms and the World Health Organization (WHO) guidelines provided. The patient agreed to provide a sample for research, but requested that their name not be disclosed in the study.

Patients with COVID-19 who have mild to moderate symptoms, no evidence of hypoxia or pneumonia, and have a medium saturation oxygen level (SpO₂) of 90% in room air typically exhibit intermediate clinical manifestations of pneumonia, including a high temperature, cough, breathing problems, but do not suffer from pneumonia with severe symptoms. Pneumonia accompanied by any of the following symptoms and clinically significant manifestations of patients with severe COVID-19: severe difficulty breathing, a respiratory rate greater than 30 breaths per minute, and SpO₂ in room air less than 90%. Among the many severe issues that COVID-19 patients have had are sepsis or septic shock,

acute pulmonary embolism, acute coronary syndrome, or ACES, and acute respiratory distress syndrome (ARDS).

2.2 Sample collection

Nasal swabs from patients exhibiting upper and/or lower respiratory tract symptoms were acquired ten days after the commencement of symptoms and placed in sterile, labeled, screw-capped tubes containing viral transport media (VTM) to preserve viral viability during transportation to a laboratory for diagnosis. Five milliliters of blood were obtained from every participant. Each blood sample was separated into two equal quantities and placed in an Ethylene Diamine Tetra Acetic acid (EDTA) and gel tube. After leaving gel tubes at room temperature (20-25°C) for thirty minutes, they were centrifuged at 6000 rpm for ten minutes at 4°C to separate the serum. The serum specimens that measure caspase-7 levels are stored at -20°C until the ELISA test. In contrast, tumor suppressor protein P⁵³ expression detection in Trizol-blood was stored at -20°C until measured via a real-time qPCR evaluation kit. Furthermore, the following laboratory tests were conducted: C-reactive protein (CRP), serum ferritin, lactate dehydrogenase (LDH), D-dimer, and complete blood count (CBC).

2.3 Biomarker Detection

Plasma D-Dimer analysis was performed using an automated protein analyzer on the first part of the blood samples, which were put in tubes containing sodium citrate and centrifuged for twenty minutes. EDTA blood and an automated hematology analyzer (ABX Micros ES 60, Horiba, USA) were used to count white blood cells (WBC: total, neutrophils, and lymphocytes). To collect serum for measuring ferritin, lactate dehydrogenase (LDH), and C-reactive protein titer, a gel tube was used, which included another portion of the blood sample, and was centrifuged for ten min. Using the Roche Cobas Integra 400 plus electrochemiluminescence immunoassay, the serum concentrations of LDH and CRP were assessed. Following the manufacturer's recommendations, the ferritin was further evaluated using a BioMerieux miniVIDAS analyzer (ELFA).

2.4 Detection of the virus

The study groups provided nasal swabs and five milliliters of blood samples. Swabs were immediately inserted into tubes containing a viral transport medium (VTM). A QIAamp Viral RNA Mini Kit (Qiagen, Germany) was utilized to isolate the viral RNA. qRT-PCR (DIAGNOVITAL SARS-COV-2 real-time PCR kit) was used to identify SARS-CoV-2, and the manufacturer's instructions were followed. The TaqPath (CE-IVD RT-PCR Kit, Thermo Fisher, Germany) detected the Omicron variant. If a patient had a cycle threshold of 36 or below in TaqPath, the COVID-19 PCR test and the ORF1ab or nucleocapsid gene targets returned positive. However, the S gene was not found, so the infection was categorized as SGTF (S gene target failure assay).

2.5 Measurement of cysteine-aspartic proteases-7 (caspase-7) level

Serum caspase-7 levels were detected by SunLong Biotech's enzyme-linked immunosorbent assay (ELISA) kits (SL3104Hu), as directed by the manufacturer. The caspase-7 assay had a detection range of 45-4000 pg/mL and 8 pg/mL sensitivity of the kit.

2.6 Gene expression for tumor suppressor P⁵³

Following the manufacturer's instructions, RNA was extracted from the tube containing blood mixed with Triazole. The TransZO1 Up plus RNA kit was utilized to extract RNA from blood samples of the study group cases. The EasyScript® two-step gDNA deletion and complementary DNA production Super-Mix synthesized complementary DNA (cDNA). A

3 µl of RNA, one microliter of (0.5 µg/µl) anchored oligo (dT) primer, and one microliter of (0.1 µg/µl) random primer were used in RT-PCR tubes; they were subsequently incubated in a thermocycler for five minutes at 65 °C and ten minutes at 4 °C. Following incubation, it was added to a final volume of 20 µl, which contained 3 µl of RNase-free water, 1 microlitters of the enzyme mix from the Easy Script® RT/RI kit, with 1 microlitters of gDNA remover, and 10 µl of EX reaction mix. It was subsequently incubated for ten minutes at 25 °C in a thermocycler, followed by a further thirty minutes at 42 °C and 5 seconds at 85 °C in the final cycle. Specific primers (designed in this study) and the TransStart® Green Qpcr Supermix kit (Transgene, China) were used to amplify cDNA. The *P⁵³* gene (forward `5-CTTCCATTGCTTTGTCCCG-3` and reverse `5-CATCTCCCAAACATCCCTCAC-3`) and GAPDH (Housekeeping genes) (forward `5-ACATCGCTCAGACACCATG-3` and reverse `5-ATACGACTGCAAAGACCCG-3`). The reaction contained master mix, SybrGreen 10 µl, 1 µl of each primer, 3 µl of cDNA, and 5 µl of nuclease-free water. Initial denaturation of amplification involved a 94 °C temperature for 30 minutes, followed by 40 cycles of 94 °C (5 seconds), 60 °C (15 seconds), and 72 °C (20 seconds). Lastly, the dissociation contained a temperature range of 55 to 95 °C and a one-minute cycle. The folding rate of *P⁵³* gene levels was calculated using the formula 1- ΔCt (each patient and healthy control sample) = Ct target gene. - Ct housekeeping gene, 2- $\Delta\Delta Ct$ (each case) = ΔCt patient sample- ΔCt average control samples, 3- Fold of the expression of genes (each sample) = $2^{-\Delta\Delta Ct}$, Folding ratio = $2^{-\Delta\Delta Ct}$ average of patients/ $2^{-\Delta\Delta Ct}$ average of controls (note: Ct =Threshold cycle).

2.7 Statistical analysis

The data used for this study were compiled using a specialized statistical software package. Interquartile ranges (IQRs), medians, and other non-parametric tests were performed when the normality tests (Kolmogorov-Smirnov and Shapiro-Wilk) were significant, with a *p*-value less than 0.001. In addition, the percentages and frequencies were used for the categorical variables, either the Pearson Chi-square test or the Fisher exact test. To estimate the validity of significant parameters, such as caspase-7 and *P⁵³*, in predicting the progression of SARS-CoV-2 disease across healthy controls. A receiver operating characteristic (ROC) curve investigation was performed to calculate the area under the curve (AUC), 95% confidence interval (CI), cut-off value, sensitivity, and specificity. This research employed logistic regression to evaluate the odds ratio (OR) and 95% confidence interval (CI) for the detection of disease severity. Patients were classified into high- and low-producer groups for caspase-7 and *P⁵³*, based on the median levels of these proteins, with a control group serving as the reference category. Spearman's rank-order correlation was used to analyze the association between caspase-7 and *P⁵³* median, as well as other studied markers in COVID-19 patients. A *p*-value ≤ 0.05 indicated a statistically significant outcome. These analyses were conducted by GraphPad Prism version 10.0 (San Diego, California, USA) and the statistical program IBM SPSS Statistics 27.0 (Armonk, NY: IBM Corp). Sample size was evaluated utilizing G*Power version 3.1.9.7, effect size (0.5), and power (1- β) of 0.91.

3. Results

3.1 Infection severity among patients with Omicron SARS-CoV-2 infection

Table 1 shows that the patients in the present study were categorized into two groups based on the severity of their infection: mild to moderate and severe to critical. The median (interquartile range, IQR) for the two groups of patients is 44.5 pg/mL (IQR: 40 - 56) and 50 pg/mL (IQR: 45 -63), respectively. This suggests that although there are no appreciable variations in the median values between these patient groups, the median value in severe-critical patients was slightly higher than that in mild-moderate patients. Additionally, our study's patients were categorized into three groups: those aged 25-39, 40-54, and 55-69 years

old. According to the statistical analysis, no notable differences ($p = 0.296$) were found between the age groups of patients with COVID-19.

Table 1: Baseline features of patients with Omicron SARS-CoV-2 categorized by the degree of infection.

Characteristic		COVID-19 Patients		p-value
		Mild-Moderate no. 61(%)	Severe-Critical no. 13(%)	
Age; year		44.5 (40 - 56)	50 (45 - 63)	$p = 0.156$
Age group	25 – 39	15 (24.6)	2 (15.4)	$p = 0.296$
	40 – 54	27 (44.3)	4 (30.8)	
	55 – 69	19 (31.1)	7 (53.8)	
	p-value	$p = 0.080$	$p = 0.232$	
Sex	Male	18 (62.2)	8 (59.1)	$p = 0.028$
	Female	43 (37.8)	5 (40.9)	
	p-value	$p = 0.001$	$p = 0.405$	
Diabetes Miletus	Yes	19 (28.9)	5 (81.8)	$p = 0.039$
	No	42 (71.1)	8 (18.2)	
	p-value	$p = 0.003$	$p = 0.405$	
Hypertension	Yes	20 (32.8)	7 (53.8)	$p = 0.152$
	No	41 (67.2)	6(46.2)	
	p-value	$p = 0.007$	$p = 0.782$	
Ct value	19 – 25	11 (18)	3 (23.1)	$p = 0.351$
	26 – 30	28 (45.9)	8 (61.5)	
	31 – 35	22 (36.1)	2 (15.4)	
	p-value	$p = 0.026$	$p = 0.092$	
CRP mg/L		13.7 (12 -14.2)	43.6 (40.3-50)	$p < 0.001$
Ferritin ng/ml		388.4 (361-446)	780.5 (660-980)	$p < 0.001$
D. dimer mg/L		279 (189-391)	1078 (900-2700)	$p < 0.001$
LDH IU/L		295(253-344)	845(679-890)	$p < 0.001$

Age data are presented as either a number and percentage (for categorical variables) or a median with interquartile range (for continuous variables); p: the likelihood of the two-tailed Fisher exact test, the Pearson Chi-square test (for categorical variables), or the Mann-Whitney U test (for continuous variables).

Results showed sex differences, with men exhibiting higher infection rates than women. Based on infection severity (mild to moderate and severe to critical, respectively), generally, the incidence of COVID-19 infection was higher in men (18, 62.2%) than in women (43, 37.8%). Additionally, based on global epidemiological data, men also had a higher percentage of illness and death than women, as shown in Table 1.

The study showed an indirect correlation between chronic conditions, such as diabetes mellitus (DM) and hypertension (HTN), and the severity of SARS-CoV-2 infections. This may be connected with a higher disease incidence ($p = 0.003$, $p = 0.007$, respectively) among patients with mild to moderate SARS-CoV-2 infections, as shown in Table 1.

Additionally, Table 1 demonstrates that the Ct values of the patients were split into three ranges: 19–25, 26–30, and 31–35. Although statistical analysis of patients indicates notable variations in percentage between cases of COVID-19 patients in mild-moderate and Ct values ($p = 0.026$), the study's findings revealed that the majority of patients (61.5%) with severe and critical situations had Ct values between 26 and 30. This explains an inverse relationship between the amount of virus present and the Ct value; therefore, the lower Ct value range corresponds to a larger viral load, and the opposite is true.

Furthermore, the severity of infection with COVID-19 was linked with a substantial difference in the median (IQR) of biomarkers CRP, ferritin, D-dimer, and LDH ($p < 0.001$), particularly in severe and critical cases, as shown in Table 1.

3.2 Cysteine-aspartic proteases-7 (caspase-7) and tumor suppressor protein (p^{53}) levels

Figure 1 reveals that caspase-7 and P^{53} levels were significantly decreased compared to controls ($p < 0.0001$), median (IQR) levels of caspase-7 and P^{53} in the patients [2907(2717-3185) pg/ml and 0.56(0.23-1.44) fold, respectively] were less than those in the group that was healthy individuals. These results indicate that the infection caused caspase-7 levels and P^{53} gene expression to be down-regulated, particularly in the initial stages of inflammation. As a result, the early stages of the disease were characterized by increased viral invasion and multiplication.

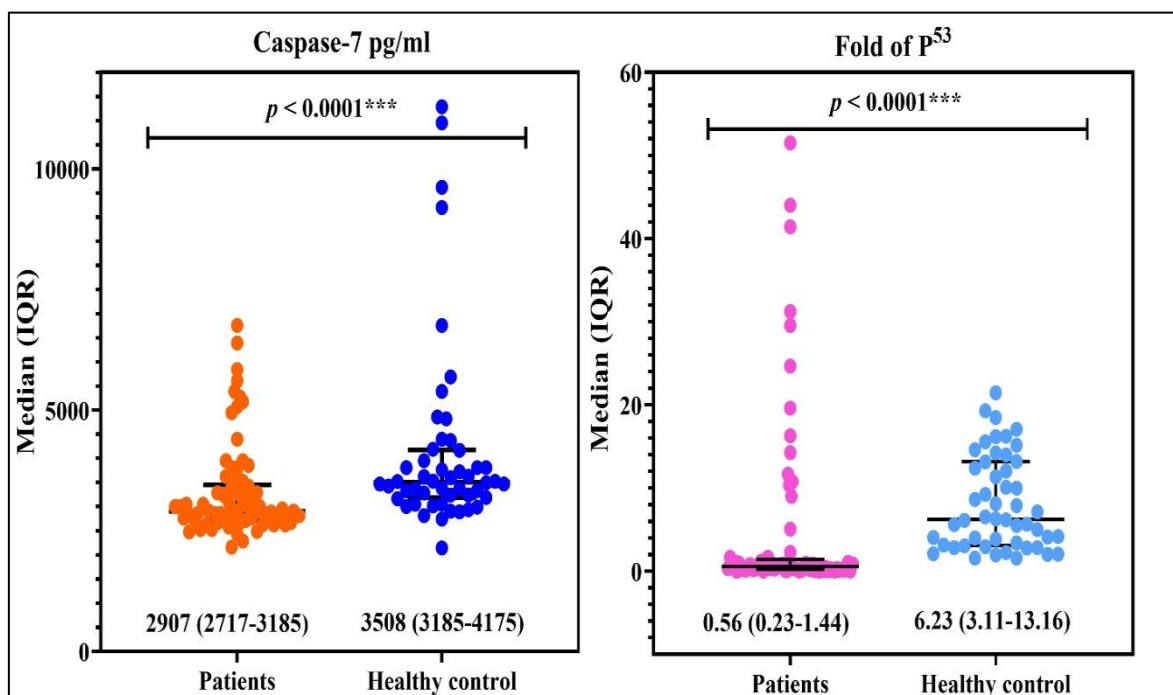


Figure 1: Median levels of caspase-7 pg/ml and P^{53} fold in COVID-19 patients and healthy controls.

To investigate whether caspase-7 and P^{53} levels were influenced by infection severity in patients with COVID-19 compared to the control group, the severity cases were stratified into mild to moderate and severe to critical categories (Table 2). More cases of mild to moderate were observed in individuals with low levels of caspase-7 and P^{53} , possibly due to downregulation of P^{53} by SARS-CoV-2 infection, especially during the early phase. This protein plays a vital role in the control and regulation of apoptosis. A substantial variation was demonstrated in severe-critical cases with P^{53} (OR=2.45 – 55.76; $p=0.001$), but no significant variation was observed among strata with caspase -7; our results exhibited elevated p^{53} expression in the severe-critical cases, and induced caspase-7 production in the outcome of SARS-CoV-2 illness.

Table 2: COVID-19 patients (mild to moderate and severe to critical cases) and controls, stratified by median caspase-7 and P^{53} level.

Groups	Caspase-7 level, pg/ml		OR	95% CI	p-value
	High (>median)	Low ($\leq$ median)			
Control	22	28	Reference		
Mild-moderate	24	37	0.83	0.39 – 1.75	0.700
Severe-critical	9	4	2.86	0.81 – 10.09	0.129
P^{53} fold					
Control	16	34	Reference		
Mild-moderate	22	39	1.20	0.55 – 2.63	0.692
Severe-critical	11	2	11.69	2.45 – 55.76	0.001

OR: Odds ratio; CI: Confidence interval; p : two-tailed Fisher's exact test (for comparing categorical variables).

3.3 ROC analysis

The receiver operating characteristic (ROC) curve analysis revealed the predictive significance of caspase-7 levels in patients of COVID-19 *versus* healthy controls (HC). The area under the curve (AUC) of caspase-7 levels is 0.790, with a 95% confidence interval of 0.692 – 0.888; $p < 0.0001$. Therefore, an AUC suggests that the caspase-7 level is a reliable predictor of SARS-CoV-2 infection, enabling the virus to overcome host defenses. The cutoff value for the caspase-7 level is more than 3169 pg/ml, with sensitivity = 76.7%; specificity = 73.3%, as shown in Figure 2.

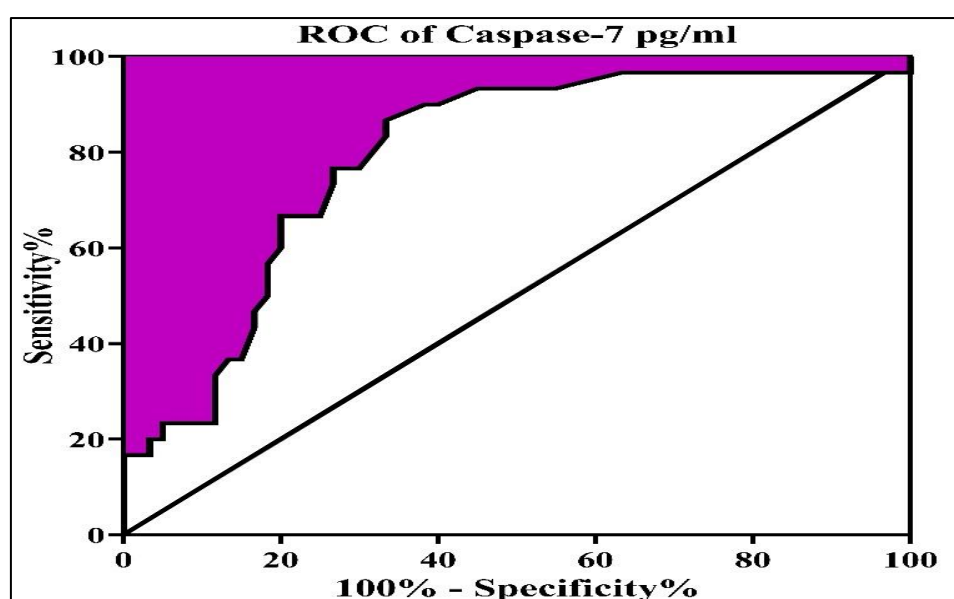


Figure 2: ROC curve analysis of caspase-7 in COVID-19 patients *versus* healthy controls (HC). A caspase-7 level of more than 3169 pg/ml can predict COVID-19 patients less risky (area under the curve = 0.790; 95% confidence interval = 0.692 – 0.888; $p < 0.0001$; sensitivity = 76.7%; specificity = 73.3%).

3.4 Correlation of caspase-7 with P^{53} , along with patient factors and biomarkers

According to Table 3, the current study's results revealed noticeable differences in caspase-7 levels among all the patient groups, regardless of age. These differences are due to lower levels in aged patients (p -value of CASP7 = 0.025). Our findings, however, revealed no discernible variations with P^{53} (p -value = 0.458).

Regarding sex, the results revealed no significant variations in CASP7 and P^{53} levels between males and females ($p = 0.184$ and $p = 0.279$, respectively). Although there are no substantial variations in caspase-7 levels between female and male patients, the levels in female patients were slightly higher than those in male patients.

Furthermore, there are two severity classifications: mild to moderate and severe to critical. There are appreciable variations in caspase-7 and P^{53} levels between severity categories. The study's findings confirmed that median levels of caspase-7 and P^{53} were higher during severe to critical infections (at 3299.9 and 2.06, respectively), but lower in mild-to-moderate cases (at 2707.4 and 0.31, respectively).

In the case of Ct value, our results exhibited a highly significant correlation between the caspase-7 and P^{53} levels and the Ct value, as shown in Table 3. Still, a lower Ct value is linked with higher viral copies in COVID-19 patients, consequently impacting the severity of disease development. These findings identified caspase-7 and P^{53} as risk indicators in patients infected with COVID-19, revealing a positive connection between them and illness severity.

Table 3: Median levels of caspase-7 and P^{53} stratified by characteristics of COVID-19 patients.

Character		Median (IQR)	
		Caspase-7 pg/mL	A fold of P^{53}
Age group	25 - 39	3762.5 (3097.4 – 4367.5)	0.77 (0.22 – 2.8)
	40 - 54	3009.9 (2670.1 – 3484.8)	0.49 (0.23 – 0.89)
	55 - 69	2859.9 (2717.5 – 3239.8)	0.29 (0.08 – 0.83)
	<i>p</i> -value	$p = 0.025$	$p = 0.458$
Sex	Male	2883.7 (2617.5 – 3407.4)	0.55 (0.26 – 1.22)
	Female	3107.4 (2764.9 – 3809.6)	0.37 (0.13 – 0.88)
	<i>p</i> -value	$p = 0.184$	$p = 0.279$
Severity	Mild-moderate	2707.4 (2517.5 – 3109.9)	0.31 (0.13 – 0.89)
	Severe-critical	3299.9 (2764.9 – 3909.6)	2.06 (0.91 – 6.12)
	<i>p</i> -value	$p = 0.018$	$p = 0.011$
Ct-Value	19 – 25	5125.1 (3239.8 – 5607.4)	24.67 (0.98 – 51.51)
	26 - 30	2965.0 (2617.5 – 3407.4)	0.41 (0.08 – 0.83)
	31 - 35	2798.7 (2670.0 – 3194.8)	0.27 (0.13 – 0.54)
	<i>p</i> -value	$p = 0.001$	$p = 0.001$

IQR: Interquartile range; NA: Not applicable; *p*: Kruskal-Wallis test and Mann-Whitney *U* test probability.

Figure 3 illustrates the Spearman rank-order correlation coefficient (r_s) for caspase-7 levels with biomarkers, including CRP, ferritin, D-dimer, and LDH levels in the patients infected with COVID-19. The results indicated no substantial association between levels of caspase-7 and CRP and LDH, while a significant association was found with ferritin and D-dimer ($p < 0.05$). Additionally, this study found a negative correlation between neutrophils and caspase-7 levels, with a significant correlation between caspase-7 levels and blood cells.

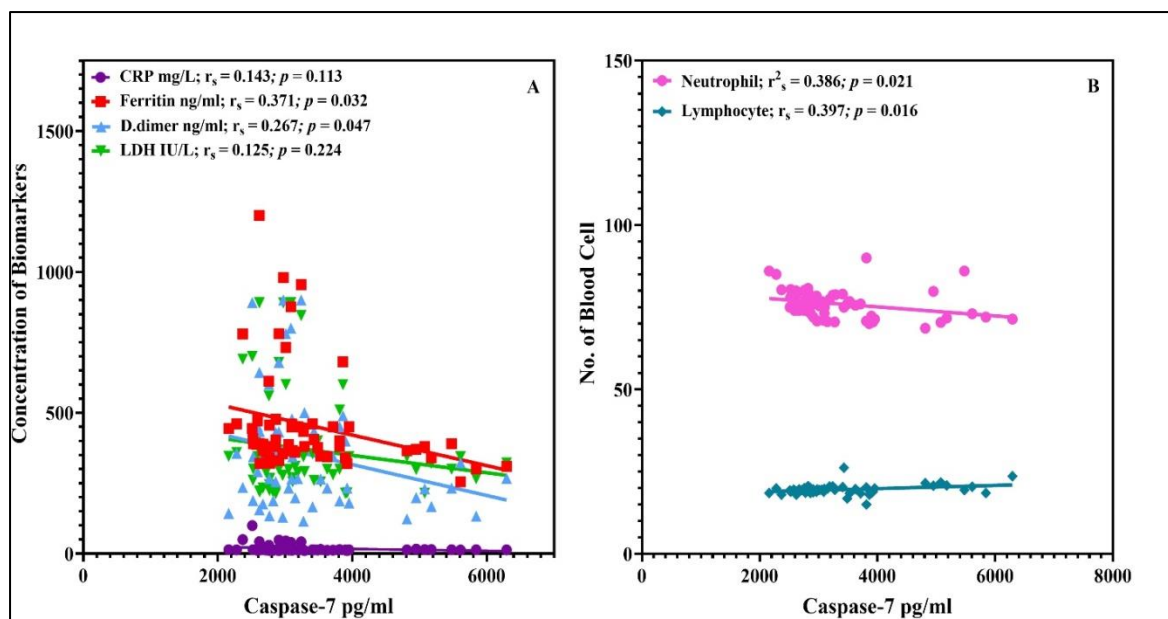


Figure 3: Correlation of caspase-7 pg/ml analysis with biomarkers and blood cells. **A:** Caspase-7 level with CRP, ferritin, D-dimer, and LDH levels; **B:** Caspase-7 level with neutrophil and lymphocyte in COVID-19 patients.

Spearman's rank association coefficient (r_s) between P^{53} gene expression and caspase-7 levels in COVID-19 patients was demonstrated in Figure 4. The results revealed a strong and substantial correlation between caspase-7 levels and P^{53} gene expression ($p = 0.0002$), suggesting that P^{53} gene expression was a crucial regulator of caspase-7 expression in patients infected with COVID-19 and that there was an advantageous connection between P^{53} and caspase-7.

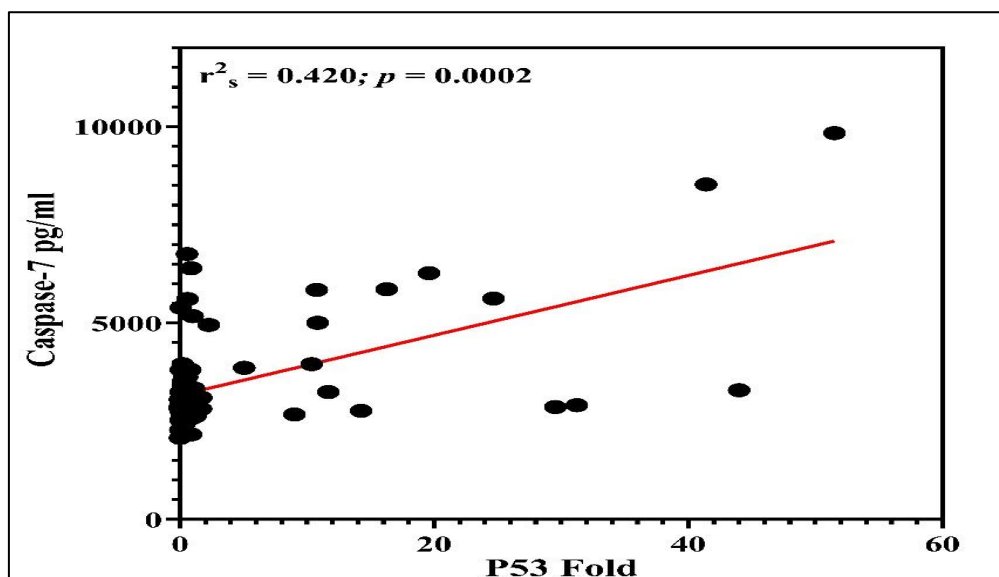


Figure 4: Correlation between P^{53} gene expression and caspase-7 level among patients.

4. Discussion

The SARS-CoV-2 virus can create new variants by altering its genomic RNA. So far, we have seen many different strains of SARS-CoV-2. Additionally, the vaccine development, which was quite successful against the early strain, led to a decline in death and illness rates. Nevertheless, Omicron, the novel strain, has exhibited certain traits, particularly its high

contagiousness, even to those who have received vaccinations or have already contracted other types of illness with varying levels of severity [13, 14].

According to our research, age is a significant independent predictor of the severity of COVID-19 illness, notwithstanding earlier studies that demonstrated a strong correlation between age and illness severity [15]. In the current study, older patients have a higher mortality rate and are more susceptible to developing acute respiratory distress syndrome (ARDS). Due to weakened immune systems, older age may be linked with increased death rates and ARDS in COVID-19. These results agree with other studies that showed Omicron infections are most evidently affecting the elderly, which has contributed to the epidemic's high death rate [16]. Moreover, our results revealed a statistical correlation between males and females, with men generally having a higher incidence of SARS-CoV-2 infection than women, based on infection severity. Therefore, most patients in this study were over 50 years old, and men comprised the largest proportion of the patient population (59.1%). Protection for females against infection may be provided by lower expression of angiotensin-converting enzyme-2 (ACE 2; coronavirus receptor) in females than in males, as well as the activity of X- chromosome-linked genes and sex-specific steroids (sex hormone), which are crucial for innate as well as adaptive immunity, may be why females are less susceptible than males to COVID-19 infections, which is consistent with earlier research studies [17]. Additionally, our findings show that older males with chronic comorbidities are more susceptible to COVID-19 infection. This may result from the weaker immune function of these patients [18]. It has been noted that a considerable proportion of younger people get severe COVID and die from it, even though age is an independent risk indicator for severe illness. This is because individuals had different immunological responses to SARS-CoV-2, or due to an excessive amount of inflammatory response, which led to a worsening of the disease and a higher death rate.

Furthermore, the study's findings showed an indirect correlation between chronic conditions, such as diabetes mellitus, hypertension, and other problems with the severity of COVID-19 infections. Still, it is more common in older adults. It has been hypothesized that older individuals with multiple comorbidities are more susceptible to the coronavirus because they are more likely to have weakened immune systems and diminished bodily functioning. These results agree with other studies that showed comorbid conditions such as obesity, diabetes, and lung illness raise the chance of unfavorable COVID-19 infections [19].

Although statistical analysis of patients indicates notable variations in percentage between case severity of COVID-19 patients and Ct values ($p = 0.02$) in mild to moderate cases, the study's findings revealed that the majority of patients (61.5%) with severe and critical situations had low Ct values. This explains an inverse relationship between the amount of virus present and the Ct value; therefore, the viral load is greater in the lower value range, and the opposite is true. This implies that assessing the viral load of SARS-CoV-2 may help determine the severity and course of the illness [20]. These findings align with a previous study that identified a negative relationship between Ct values and disease severity, and viral load, as well as with other studies that have shown the omicron variant to have a non-significant relationship with age, Ct value, and infection severity [21].

C-reactive protein levels in the blood are elevated due to inflammation, bacterial or viral infections, or tissue damage. The CRP indicates inflammation. A high CRP level typically accompanies a systemic inflammatory condition, such as severe COVID-19 cases, as well as a surge of cytokines resulting from the massive release of inflammatory cytokines, similar to Previous studies. The LDH level was elevated in COVID-19 patients, particularly in those with severe to critical disease. Earlier studies in various parts of the world have yielded similar findings. Other researchers have found that higher levels of LDH and other pro-inflammatory indicators, including CRP and IL-6, as well as TNF, are often linked to worse

outcomes, and our findings align with those of previous research [22]. Ferritin is a complex of globular proteins comprising 24 subunits, and it is the principal iron storage protein in the inner cells. It maintains iron in a soluble and non-toxic state. Because iron and its binding proteins have crucial immune regulatory properties, iron overload, a significant side effect of both SARS-CoV-2 and transfusion treatment, is believed to be the primary precipitating mechanism in pathogenesis. Excess iron can alter the immunological balance and promote the proliferation of infectious microbes, and it is probably necessary for the replication and the function of SARS-CoV-2 viral RNA [23].

Historically, caspases have been categorized into two major groups: inflammatory and apoptotic, based on the cellular processes they support. Inflammatory caspases, including caspase-1, -4, -5, and -11, cause inflammation by triggering pyroptosis and the inflammasome. On the other hand, effector caspases, such as caspase-3, -6, and -7, and apoptotic caspases, such as caspase-2, -8, -9, and -10, participate in immune suppression [24]. Additionally, most RNA viruses, including SARS-CoV-2, have developed mechanisms to prevent or postpone apoptosis, as numerous viral proteins that directly interact with host protein caspases have been shown to favorably or negatively affect apoptosis. Under this concept, several virus species encode proteins that interfere with caspase-7 thereby inhibiting its proapoptotic signaling. This gave the virus more time to proliferate and assemble its particles, resulting in a persistent viral infection [25]. These findings are consistent with our observation of decreased caspase-7 levels, an apoptotic effector caspase, as shown in Figure 1. This decrease in the levels of caspase-7 in patients may result in downregulation or shutdown of gene expression of caspase-9 (one of the initiator caspases) via inhibitors that are released by the virus. According to an earlier study, hepatitis B's viral encoded protein, HBx (hepatitis B Virus X protein), has a detrimental effect on producing p^{53} -induced apoptosis by blocking caspase activation, enabling the infected cells to survive longer and continue producing viral particles [26]. In contrast, many viruses, including HIV, influenza, and hepatitis C (HCV), can activate apoptotic pathways to eliminate infected cells and control infection [27].

The P^{53} level was noticeably lower in patients with COVID-19 compared to healthy individuals and other biomarkers. In the initial stages of infection, the viral proteins inhibit p^{53} expression, which stops replication and sets off an inflammatory reaction. Its decline was partly caused by poor interferon gene expression, which resulted in decreased p^{53} levels, thereby delaying apoptosis [28]. These results agree with our results that showed a decrease in levels of P^{53} in individuals with SARS-CoV-2 omicron infections compared to healthy people ($p < 0.0001$), as shown in Figure 1. The P^{53} is considered the link between coronavirus infection and apoptosis, which cannot be ignored. Interestingly, p^{53} has the same direct regulatory effect on SARS-CoV-2 replication as other coronaviruses. Another significant way that SARS-CoV-2 and p^{53} interact is through upregulating the primary p^{53} regulator, murine double minute 2 (MDM2) proteins [29].

Furthermore, the study found that more cases of mild to moderate disease were observed in individuals with low levels of caspase-7 and P^{53} , as shown in Table 2. Caspase-7 low levels may be due to the inhibition of Caspase-9 and 8 by SARS-CoV-2 infection, especially in the early phase of it. These caspases play an essential role in activating effector caspases such as caspase-7 and 3, which are necessary for apoptosis. A recent study showed that to execute apoptosis, the activated caspase-9 further processes and activates the downstream effector caspases of caspase-3 and caspase-7 [30]. But, caspase-9 may play a double role in viral infections. It can both participate in the host's defense by promoting cell death of infected cells or be targeted by viruses to evade immune responses. Growing evidence suggests that apoptosis is essential in combating infection by eliminating infected cells, thereby protecting the host. However, the lysis of these cells results in the creation of DAMPs

and cytokines, leading to a hyper inflammatory response with the discharge of cytokines that promote inflammation, rather than the silent death caused by apoptosis. Moreover, caspases 9 and 7 are activated when mitochondrial proteins, such as cytochrome c, are released, which occurs as a result of mitochondrial apoptosis in the severe stages of infection [31]. This finding aligns with a previous study that demonstrated that inhibiting caspases and apoptosis may result from elevated levels of TNF- α that increase superoxide production. Our results exhibited slightly elevated caspase-7 levels in the severe-critical cases. This result aligns with a previous study. Plassmeyer *et al.*, observed that, compared to healthy controls, RBCs from severe COVID-19 exhibited elevated caspase-3/7 activity [32]. Typically, p^{53} is kept at low levels in healthy physiological mammalian cells, often by ongoing ubiquitination. Still, subsequent degradation by the main p^{53} regulator murine double minute 2 (MDM2) proteins, at varying concentrations, may suppress p^{53} in different ways. According to earlier research, the SARS-CoV-2 virus triggers MDM2 expression and suppresses p^{53} expression, especially in the early stage of infection. As a result, p^{53} is degraded, which prevents p^{53} -mediated apoptosis and encourages viral growth [33]. The MDM2 can inactivate p^{53} in various ways, depending on its concentration. Consequently, MDM2 levels are typically inhibited in conjunction with p^{53} activation. Nevertheless, p^{53} ubiquitylation is suppressed in response to viral infection, hypoxia, or DNA damage, which permits it to build up in the nucleus. Phosphorylation and acetylation are two of the several covalent modifications that p^{53} undergoes, activating and stabilizing the protein, especially in cases of severe infection [34]. This finding aligns with our study's results, which demonstrated that more cases of mild to moderate disease were observed with low levels of P^{53} , with elevated p^{53} levels in the severe-critical instances, as shown in Table 2. A complicated and situation-specific connection exists between p^{53} and apoptosis during coronavirus infection. Certain coronaviruses can alter the p^{53} pathway to prevent apoptosis and encourage their replication, even though p^{53} can encourage apoptosis as a host defensive mechanism against viral infection. This shows the dual role of p^{53} in regulating apoptosis during SARS-CoV-2 infection [35]. This finding aligns with the results of our study, demonstrating a positive correlation between caspase-7 levels and P^{53} gene expression.

5. Conclusions

This study revealed that caspase-7 and P^{53} expression levels in patients with SARS-CoV-2 decreased due to elevated viral loads, irrespective of factors such as age, and sex. Reduced levels of caspase-7 and P^{53} are associated with the early phases of SARS-CoV-2 infection. Due to the impact of the Omicron variant on the genes that regulate P^{53} expression, leading to suppression and inhibition of apoptosis pathways. Moreover, the results revealed a beneficial connection between P^{53} and caspase-7, suggesting that P^{53} gene expression was a crucial regulator of caspase-7 increase expression in patients infected with the SARS-CoV-2 Omicron variant in the severe phases. As a result, the results showed a negative correlation between viral load and caspase-7 and P^{53} expression, while both caspase-7 and P^{53} were positively correlated with the progression of SARS-CoV-2 infection. Therefore, research investigating the activity levels of caspase-7 and its interaction with P^{53} during viral infections is essential for fully understanding this subject's inherent potential and characteristics.

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7. Ethics approval and consent to participate.

This research occurred between February and November 2024. All participants provided informed written permission. The ethics committee of the Department of Biology in the College of Science, University of Baghdad, accepted the study (reference code CSEC/0225/0025).

8. Disclosure and conflict of interest

The authors affirm that they have no competing interests.

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