The Egyptian Journal of Hospital Medicine (October 2022) Vol. 89, Page 5392- 5399

Relationship between Caspase-8 and SARS-CoV-2 Variants in a Sample of Iraqi Population

Jinan J. Ghazzi*, Hula Y. Fadhil

[1] department of biology, college of science, university of Baghdad, Baghdad, Iraq

* Corresponding author: Jinan J. Al-Kafagi**, Mobile:** (+964) 7741786166, **E-Mail:** jaliljinan1@gmail.com

ABSTRACT

Background: Mutations started to accumulate in SARS-CoV-2 due to replication errors leading to emergence of variants. New variant appeared in December 2020. Caspase-8 can process pro-IL-1 and IL-18 causing the release of bioactive cytokines through immunogenic cell death that can kill virus-infected cells and activate the innate and adaptive immune systems. **Objective:** This study aimed to find the relationship between caspase-8 levels and wild-type, alpha, beta or gamma and omicron variants and its correlation with severity, CRP, and WBC.

Materials and methods: 134 nasal swabs and blood samples were collected from COVID-19 patients and 48 healthy individuals. RNA was extracted and rRT-PCR assay to detect variants using a special kit**.** Caspase-8 levels were determined by ELISA. **Results:** There was a significant relationship ($p < 0.001$) in patients median caspase-8 than in control. Logistic regression analysis showed that odds ratio (OR) of unadjusted patients was 4.33 and with age-adjusted was 7.24. Caspase-8 levels showed a significant relationship with the severity of infection by COVID-19 ($p < 0.01$) and highly significant relation with variants of SARS-CoV-2 ($p < 0.0001$), as follows: Alpha, wild type, beta or gamma and omicron. Caspase-8 levels were significantly increased with increase of CRP levels ($p < 0.05$) making both of them good prognostic markers for infection progress. Observed negative Spearman correlation (rs = -0.27; $p < 0.01$) were detected between caspase-8 and WBC.

Conclusion: The most severe infections with SARS-CoV-2 appeared in wild type and alpha variant. Caspase-8 and CRP are excellent biomarkers for the progression of infection.

Keywords: SARS-CoV-2, Caspase-8, rRT-PCR, COVID-19, ELISA.

INTRODUCTION

On December 31, the Wuhan Municipal Health Commission made a pneumonia outbreak announcement on their website. Later, researchers revealed the discovery of a previously unidentified coronavirus [(1)]. World Health Organization (WHO) designated the illness as coronavirus disease 2019 (COVID-19), and the International Committee on Taxonomy of Viruses (ICTV) designated the new infectious agent as severe acute respiratory corona virus-2 (SARS-CoV-2) [(2)]. The first confirmed SARS-CoV-2 case was reported in Iraq on February 24, 2020, by an Iranian student. Since then, the number of cases has climbed both domestically and globally [(3)].

Rapid global spread of the SARS-CoV-2 resulted in several variants of concern (VOCs), which finally developed into a serious and lethal outbreak [(4)].Unsurprisingly, as the number of COVID-19 instances rose, mutational differences that may have a greater influence on fitness, such as those that could result in immunological escape, also occurred. In actuality, there are likely escape mutations in the ten human monoclonal antibodies that target the SARS-CoV-2 RBD [(4)]. A novel SARS-CoV-2 variant of concern, the Alpha variant, was discovered in the UK in late December 2020 based on whole-genome sequencing of samples from patients who tested positive for SARS-CoV-2 [(5)]. The alpha form of the viral genome carries 17 mutations. There are eight of these mutations in the spike (S) protein. In Nelson Mandela Bay, South Africa, a second wave of COVID-19 infections was brought on in October 2020 by a novel SARS-CoV-2 lineage, the Beta variant [(6)].

The Beta form of the spike protein carries nine mutations. The third variant of interest is Gamma variant. It was first seen in the US in January 2021 after being found in Brazil in December 2020. There are ten mutations in the variant's spike protein. This variant has spread to 45 nations, according to the WHO epidemiological bulletin from March 30, 2021 [(7)].

The WHO has named the most recent variant of concern as the Omicron variant. It was discovered for the first time in South Africa on November 23, 2021, following an increase in the number of COVID-19 patients. Along with the high increase in cases seen in South Africa, Omicron had more than 30 mutations to the virus' spike protein [(8)].

Caspase-8 has been discovered to be a master regulator of the three primary cell death pathways, including apoptosis, pyroptosis, and necroptosis. Previously, it was only recognized as an apoptotic caspase [(9)]. Recently, it was found that caspase-8 can function similarly to caspase-1 in processing pro-IL-1 and IL-18, releasing bioactive cytokines through either pyroptosis or necroptosis and triggering the generation of pro-inflammatory cytokines. Necroptosis, an immunogenic cell death process, can kill virus-infected

Received: 17/06/2022
Accepted: 25/08/2022

cells while triggering the innate and adaptive immune systems to halt the virus from spreading. [10].

However, occasionally the advantages of necroptosis to the host may be outweighed by the potentially hazardous hyper-inflammatory effects of initiating this death pathway in pulmonary and other tissues [11]. For instance, necroptosis in airway epithelial cells brought on by influenza A virus (IAV) infection is connected to lung damage and excessive inflammatory responses. Like IAV, SARS-CoV-2 can cause severe lung damage and disease-related hyper-inflammatory responses. [12].

MATERIALS AND METHODS

134 patients between the ages of 17 and 68 who were suspected of having the SARS-COV-2 virus had their nasal swabs and entire blood samples taken, with the vaccinated patients being excluded. In order to detect the level of caspase-8, 48 swabs and blood samples were also taken from healthy persons. RNA from viruses is extracted using a nucleic acid extraction kit (magnetic beads method, zybio manufacturers, China). Using a specialist SARS-COV-2 detection kit (Accupower® SARS-COV-2 multiplex real time RT-PCR kit, Bioneer, Korea), reverse transcriptase real-time PCR (rRT-PCR) test, and to validate the diagnosis of SARS-COV-2 infection and calculate cycle threshold (Ct) value. Detection of SARS-COV-2 variants (Alpha, Beta/Gamma) and wild-type by using rRT-PCR assay and using Accu-power® SARS-COV-2 variants ID 1 kit (Bioneer, Korea). Diagnosis of omicron was performed by using the TaqPath (TaqPATH COVID-19 CE-IVD RT-PCR Kit, thermo-fisher, Germany) COVID-19 PCR test. Infections were classified as SGTF (s gene target failure assay) when a patient's TaqPath COVID-19 PCR test was positive, and the ORF1ab or nucleocapsid gene targets had a cycle threshold of 36 or fewer, but the S gene wasn't detectable [13]. A human caspase-8 ELISA kit from Al-Shkairate establishment for medical supply in Jordan was used to measure the amount of caspase-8 in the serum of 48 healthy control cases and 134 patients with specific conditions (32 wild-type, 22 Alpha, 38 Beta or Gamma, and 46 Omicron). Caspase-8 was detected in serum samples using the ELISA method. Standard had a detection range of 0.156–10 ng/ml and a sensitivity of 0.094 ng/ml. Additionally, the Roche Cobas Integra 400 plus and the ABX Micros ES 60, Automated Hematology Analyzer, respectively, were used to examine the CRP and WBC count.

Ethics approval:

An approval of this study was obtained from the University of Baghdad Academic and Ethical Committee (CSEC/0921/0042). Informed consents of all the patients were taken. This study was carried out in accordance with the World Medical Association Code of Ethics (Declaration of Helsinki) for studies involving humans.

Statistical Analysis

The statistical software packages GraphPad Prism 8.0.0 and IBM SPSS Statistics 25.0 (IBM Corp., Armonk, NY) were used to manage and conduct these analyses on frequencies and percentages. The Mann-Whitney U and Kruskal-Wallis tests were used to determine whether there were significant differences between medians. Continuous variables were first evaluated for normality using a normality test to characterize the median with an interquartile range for not normally distributed data. The two-tailed Fisher exact test or Pearson Chi-square test was used to compare the frequency of the categorical variables, which are provided as numbers and percentages. Statistical significance was defined as a probability (p)value 0.05. In order to evaluate the validity of the significantly different parameters, such as a variant of SARS-CoV-2 across the disease severity in the prediction of severe or critical disease, receiver operating characteristics curve (ROC) analysis was used. This method calculates the area under the curve (AUC), 95% confidence interval (CI), cut-off value, sensitivity, and specificity. The AUC was calculated and is a measure of a test's validity; an AUC of less than 0.600 indicates a test's inadequacy as a predictor, while an AUC of 0.600 to 0.700 is a sufficient predictor, while an AUC of 0.700 to 0.800 is good, 0.800 to 0.900 is very good, and an AUC of more than 0.900 indicates an excellent predictor test. The Youden J statistic, also known as the Youden Index, which measures the efficacy of a dichotomous diagnostic test, was used to determine the ideal cutoff point. To determine the odds ratio (OR) and 95% confidence interval (CI) for the two models I (chronic disease) and II. Logistic regression analysis was used (dread disease). According to the mean in the without group (and > mean, respectively), patients were divided into with and without groups in this analysis, with the without group serving as the reference group. Statistical significance was defined as probability (p) 0.05.

Under three models—I (unadjusted), II (age-), and III—logistic regression analysis was used to compute odds ratio (OR) and 95% confidence interval (CI) (age and gender-adjusted). According to a median of caspase-8, patients and hypertension were divided into low and high production groups in this analysis (and > median, respectively), with the high production group serving as the reference group.

The correlation coefficient between caspase-8 and biomarkers (CRP and WBC count) in COVID-19 patients was examined using Spearman's rank-order correlation. For statistical analysis, GraphPad Prism version 8.0.0

(San Diego, California, USA) and IBM SPSS Statistics 25.0 (Armonk, NY: IBM Corp.) were both utilized. The power of the sample size was determined using the G*Power version 3.1.9.2 software [kind of power analysis: compromise; error probability: 0.05; power (1-error probability): 0.80; effect sized: 0.55; actual power: 0.80].

RESULTS

The patients who took part in the current investigation were divided into two age groups, as shown in table (1), 54 (IQR: 51-59) ≤ 45 years old and 38 (IQR: 30.5-42) >45 years old. According to the statistical analysis, which showed highly substantial (p< 0.0001) differences between the age group of COVID-19 patients, age is a significant risk factor.

Table (1): Baseline characteristics of patients with SARS-CoV-2 infection

Characteristic		SARS-CoV-2 Cases; n = 234	*p*-value
Age; year	≤ 45	38 (30.5 – 42)	< **0.0001**
	> 45	54 (51 – 59)	
Gender	Male	166 (70.94)	< **0.001**
	Female	68 (29.06)	
Ct threshold values	11 – 20	138 (58.97)	< **0.01**
	21 – 36	96 (41.03)	
Severity group	Mild-moderate	96 (41.03)	< **0.001**
	Severe	88 (37.6)	
	*Critical	50 (21.37)	
SARS-CoV-2 Variants	Wild type	32 (13.7)	< **0.001**
	Alpha	109 (46.6)	
	Beta or Gamma	47 (20.1)	
	Omicron	46 (19.6)	

Median levels of caspase-8 stratified according to characteristics of COVID-19 patients and healthy controls

As shown in table (2), there was no significant difference in the amount of the caspase-8 protein between the patient and control groups when it came to age. Older patients > 45 years had median levels of caspase-8 of 0.68 (IQR: 0.37-0.97) (p = 0.36), compared to the healthy control group's 0.62 (IQR: 0.41-0.71), and patients younger than 45 years had the median level of 0.56 (IQR: 0.41-0.79). Due to the different sample sizes between the patient and the healthy control, the median of caspase-8 in the age categories of the patient and the control groups was not statistically significant.

Table (2): Median levels of caspase-8 stratified according to characteristics of COVID-19 patients and healthy controls

Caspase-8 median (IQR); ng/mL Character (no. 122)		Control (no. 48)	Patient
Age group	≤ 45	0.56 (0.41 - 0.79)	.58 (0.45 – 0.63)
	> 45	0.68 (0.37 – 0.97)	.62 (0.41 – 0.71)
	p-value	p = 0.36	p = 0.93
Gender	Male	0.61 (0.39 – 0.9)	.56 (0.38 – 0.62)
	Female	0.78 (0.49 – 1.62)	625 (0.53 – 0.72
	p-value	< **0.05**	< **0.05**
DM	Yes	0.68 (0.37- 0.97)	NA
	No	0.62 (0.42 – 0.83)	
	p-value	p = 0.95	
HTN	Yes	0.68 (0.37 – 1.0)	NA
	No	0.62 (0.37 – 0.85)	
	p-value	p = 0.68	
Other Disease	A	0.69 (0.41 – 0.97)	NA
	B	0.44 (0.36 – 0.61)	
	C	0.51 (0.28 – 0.56)	
	D	0.65 (0.29 – 1.0)	
	p-value	p = 0.081	
Severity	Mild-moderate	0.61 (0.37 – 0.76)	NA
	Severe	0.8 (0.54 – 1.45)	
	Critical	0.34 (0.27 – 0.67)	
	p-value	< **0.01**	

Median of C-reactive protein stratified to age, gender, chronic disease and infection severity

Figure (1) showed that the median C-reactive protein had a significant relationship with the age groups of the patients who were chosen (p 0.001), with 54 patients aged 45 having the median CRP of 11.30 (IQR: 4.100-27.30) and 68 patients aged > 45 having the median CRP of 29.75 (IQR: 13.38-66.88). In comparison with younger people, elderly adults had higher median values. Gender, however, did not significantly affect CRP median (p = 0.305), with 43 females and 79 men both having a median of 20.10 (IQR: 6.300-53.10) and (IQR: 11.30-66.80).

Chronic disease (HTN, and renal failure) had a highly significant relationship with CRP median (p < 0.001). 14 patients had no chronic disease and had a median CRP of 53.40 (IQR: 29.70-83.38), whereas 108 patients had a median CRP of 17.70 (IQR: 7.200-51.43). Patients with chronic disease who were infected also had higher median CRP levels than healthy patients. CRP median had a very strongly significant connection with COVID-19 infection severity (p < 0.0001); it was 8.300 in patients with light to moderate infection, 57.20 in patients with severe infection (IQR: 33.10-71.78), and 78.20 in patients with critical infection (IQR: 76.34-98.90). Critical infection had the highest median CRP level, then severe infection, and then mild to moderate infection.

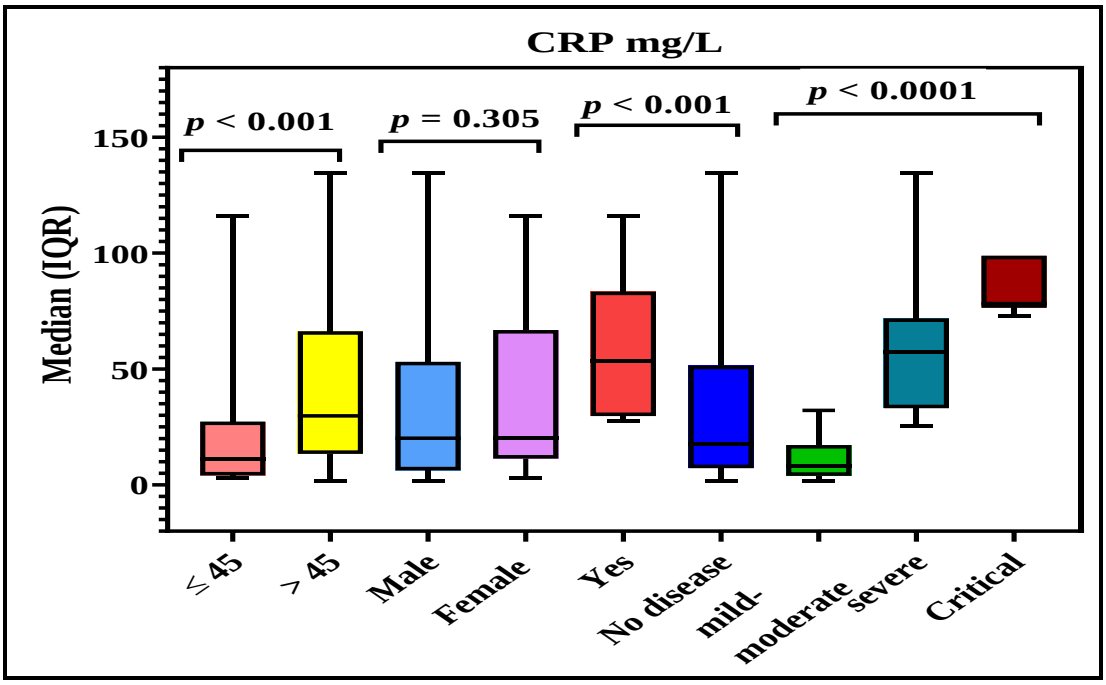

Figure (1): Median of C-reactive protein stratified by age group (≤ 45 and > 45 years), gender (male and female), chronic disease (HTN, DM and renal failure), and no disease in COVID-19 patients.

Median of caspase-8 in COVID-19 patients, healthy control and SARS-CoV-2 variants

The caspase-8 median was shown in Figure (2) stratified by SARS-COV-2 variations, COVID-19 patients, and healthy controls. The patient (122) group performed better than the control (48) group in terms of caspase-8 median ($p< 0.001$). The alpha variation of SARS-COV-2, which affected 22 patients, had the greatest median level of caspase-8, followed by the wild-type, which affected 16 patients, the beta or gamma, which affected 38 patients, and most recently the omicron, which affected 46 patients.

Figure (2) showed the caspase-8 median stratified by COVID-19 patients, healthy control and SARS-CoV-2 variants. Caspase-8 median was significantly correlated ($p < 0.001$) with the patient (122) higher than control (48) groups who were in good health. Caspase-8 demonstrated a highly significant relationship with variants of SARS-COV-2 ($p < 0.0001$), where the alpha variant 22 patients being the highest level of the caspase-8 median, followed by the wild-type 16 patient, then the beta or gamma 38 patients and lately the omicron 46 patients. According to those findings indicated that the caspase-8 is a fantastic biomarker for the infection with various variants of COVID-19 in the study as well as an indicator for progression of infection.

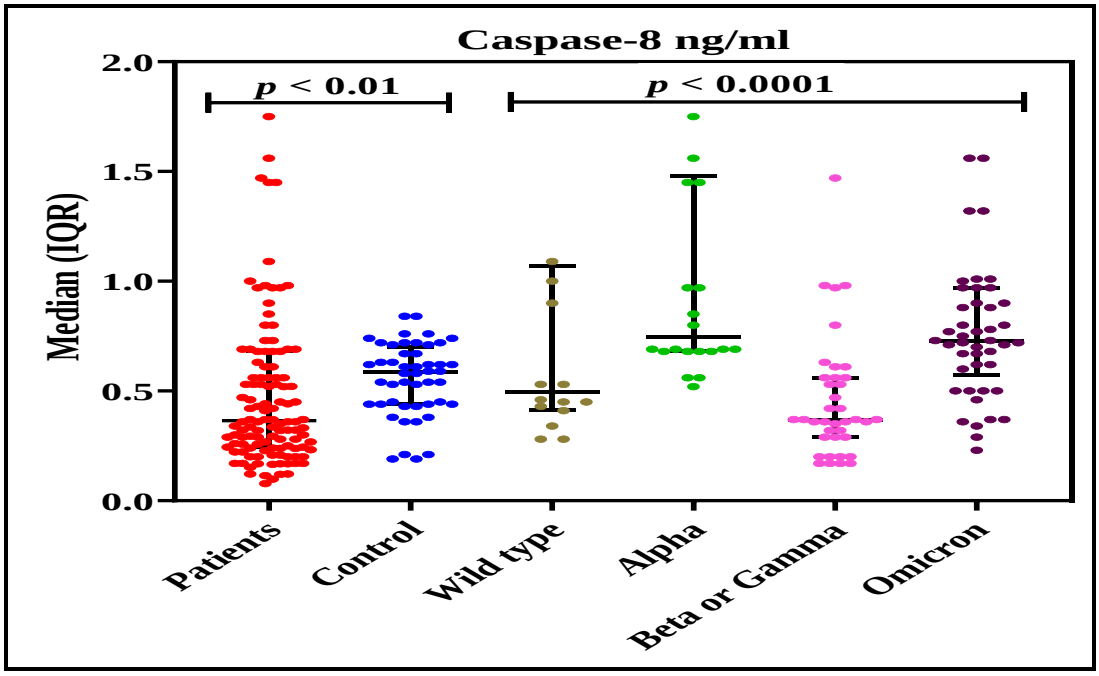

Figure 2: Scatter-dot plot of caspase-8 median stratified by COVID-19 patients, healthy control and SARS-CoV-2 variants

Correlation of caspase-8 along with WBC count and CRP

Figure (3) explained the Spearman's rank correlation coefficient (rs) between caspase-8 and CRP in COVID-19 patients. The findings showed that there was a slight positive correlation between caspase-8 and CRP (rs= 0.21). The results of the current investigation showed that the CASP-8 levels were significantly increased with the increase of CRP levels ($p < 0.05$) making both of them as a useful prognostic indicator for the development of infection.

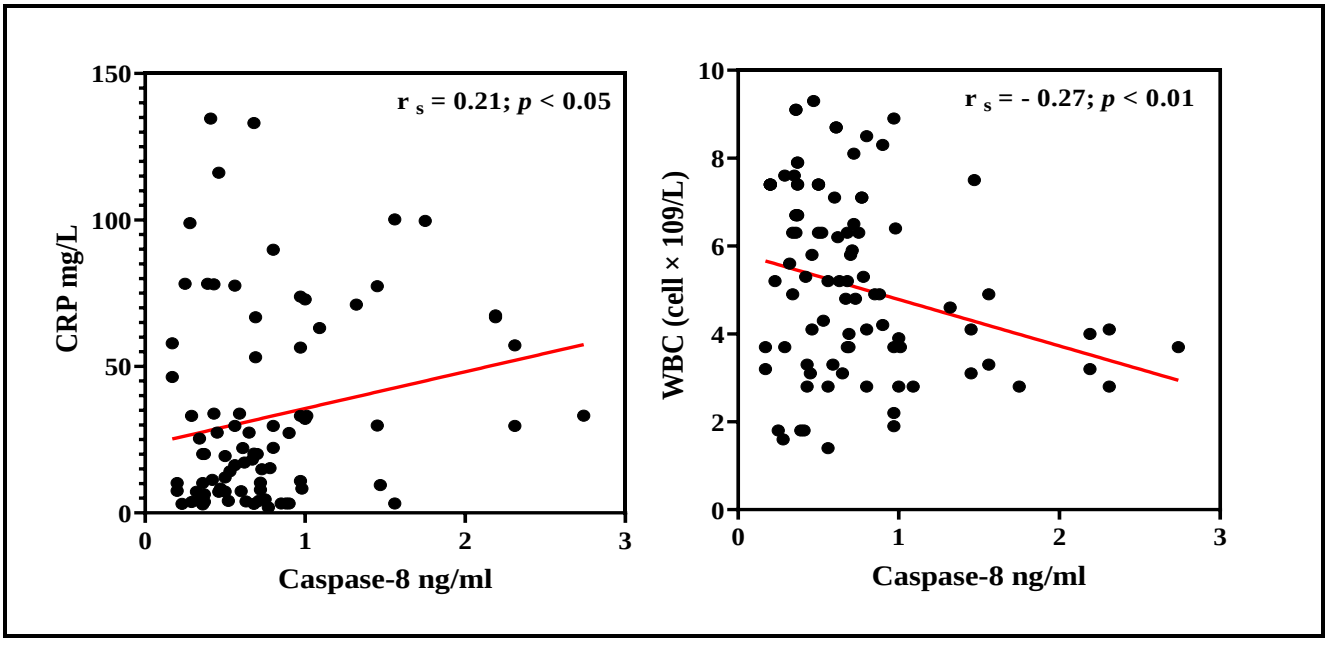

Figure 3: Spearman's rank correlation coefficient (rs) between caspase-8 along with WBC count and CRP in COVID-19 patients

Multinomial logistic regression and ROC curve analysis of caspase-8 in COVID-19 patients

According to table (3), the odds ratio (OR) of unadjusted patients (model I: age and gender were taken into account) was 4.33. This finding suggests that when caspase-8 levels increase during infection, the severity of the infection would progress more quickly. Models II and III of multinomial logistic regressions OR results were convergent at 7.2, showing that age and gender had a substantial impact on the amount of caspase-8 during infection and that this rise was correlated with these factors.

Table (3): Logistic regression analysis of caspase-8 in COVID-19 patients versus control group

Model†	OR	95% CI	*p*-value
I (unadjusted)	4.33	1.39 –13.56	**0.012**
II (age adjusted)	7.24	1.63 –32.12	**0.009**
III (age and gender adjusted)	7.22	1.62 –32.25	**0.01**

†: The reference category is > Median; OR: Odds ratio; CI: Confidence interval; p: Probability (significant p-value is indicated in bold)

The cases were divided into severity groups and a healthy control group to investigate whether the caspase-8 serum level was influenced by any of the characteristics in COVID-19 cases.

Receiver-operating characteristic (ROC) analysis is a useful technique for evaluating the efficacy of diagnostic procedures and, more generally, for the precision of a statistical model (e.g., logistic regression). The predictive value of the caspase-8 area under the curve was demonstrated by ROC curve analysis (AUC = 0.88; 95% CI = 0.824-0.936; cut-off value = 1.12 ng/ml; sensitivity = 86.07%; specificity = 87.5%). Results showed that the amount of caspase-8 during COVID-19 infection was a highly accurate biomarker for the progression of infection (Figure 4).

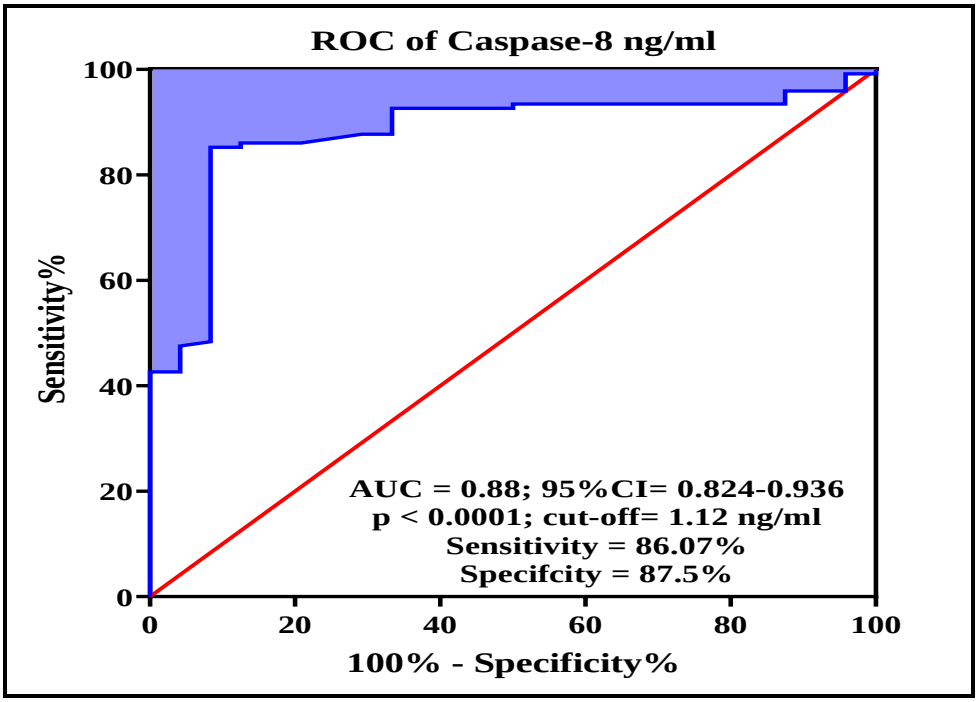

Figure (4): ROC curve analysis of caspase-8 in COVID-19 patients with severe and critical infections versus control group to predict the progression of disease (AUC: area under curve)

DISCUSSION

Baseline characters of COVID-19 patients

The patients who took part in the current investigation were divided into two age groups, 54 (IQR: 51-59) ≤ 45 years old and 38 (IQR: 30.5-42) >45 years old. According to the statistical analysis, which showed highly substantial ($p < 0.0001$) differences between the age group of COVID-19 patients, age is a significant risk factor. This result seems to be in line with more recent studies that demonstrate a causal connection between aging and the COVID-19 infection [(14)].

According to epidemiological findings from around the world, there was a larger incidence of COVID-19 infection in males 166 (70.94%) than in females 68 (29.06%). Males also had a higher morbidity and fatality rate than girls. Therefore, the variable expression of ACE2 that sex hormones generate across the body may make it simpler to identify the sex differences in COVID-19 data. Data suggest that higher levels of expression and activity of ACE2 may make it more vulnerable to COVID-19 infection and death. For instance, one study found that the lungs of patients with comorbidities associated with a higher risk of COVID-19 infection expressed ACE2 at high levels [(15)].

The patients' Ct values were divided into two categories; around 138 patients (58.97%) lie within the 11–20 range, while 96 patients (41.03%) fall within the 20–36 range. Statistical analysis revealed a significant difference between the two Ct value ranges (p 0.01). Since the viral load is higher in the lower value range and vice versa, the Ct value and viral load have an inverse relationship. Research from the past has shown that chronically high viral loads are associated with more severe diseases [(16)]. Participants in the study were divided into three groups based on the severity of the illness, as reported by the WHO: 96 (41.03%) mild to moderate, 88 (37.6%) severe, and 50 (21.37%) critical cases. Low Ct value thresholds were common among the study's severe and critical patients. Due to the time of sample collection (march 2021), when the alpha variation predominated over the wild-type, the current study's findings regarding infection with COVID-19 variants revealed that the alpha variant had the greatest infection percentage of 109 (46.6%). The beta or gamma variants were 47 (20.1%), the omicron variant was 46 (19.6%), and lastly the wild-type 32 (13.7%) were placed after this. Statistical analysis revealed a significant difference between the two Ct value ranges (p 0.01). Since the viral load was higher in the lower value range and vice versa, the Ct value and viral load have an inverse relationship.

Median levels of caspase-8 stratified according to characteristics of COVID-19 patients and healthy controls

There was no significant difference in the amount of the caspase-8 protein between the patient and control groups when it came to age. Older patients > 45 years had median levels of caspase-8 of 0.68 (IQR: 0.37-0.97) (p = 0.36), compared to the healthy control group's 0.62 (IQR: 0.41-0.71), and patients younger than 45 years had the median level of 0.56 (IQR: 0.41-0.79). Due to the different sample sizes between the patient and the healthy control, the median of caspase-8 in the age categories of the patient and the control groups was not statistically significant.

In terms of gender, the results showed a correlation between the levels of caspase-8 in male patients and female patients of 0.61 (IQR: 0.39 - 0.9) and 0.78 (IQR: 0.49 - 1.62) respectively. This suggests that female patients had higher amounts of caspase-8 than male patients. The control group likewise demonstrated that females had greater levels of the aforementioned protein, 0.625 (IQR: 0.53 - 0.72) than males. Both the patient and control groups' levels of caspase-8 were significantly influenced by gender, with a difference in males of 0.56 (IQR: 0.38 - 0.62) (p 0.\05). The findings of this study showed that caspase-8 had an indirect correlation with comorbidity, with values for patients with HTN and chronic diseases DM being 0.68 (0.37-0.97) and 0.95 (0.95-0.97) respectively. Conversely, values for infections with cardiac disease B were 0.44 (0.36-0.61), 0.51 (0.28-0.56), and 0.65 (0.29-1.0) in leukemia patients, respectively. Furthermore, there was a significant association between caspase-8 and the severity of COVID-19 infection (P 0.01), as shown by the following: For low to moderate infection severity was 0.61 (IQR: 0.37 - 0.76), for severe infection was 0.8 (IQR: 0.54 - 1.45), and for critical infection was 0.34. The results showed that, whereas levels of caspase-8 were lower during severe infection, they were higher during critical and mild to moderate infections (P 0.01). Necroptosis, an immunogenic cell death procedure, can kill virus-infected cells and trigger both innate and adaptive immune responses to prevent the virus from spreading. [(11)].

Median of C-reactive protein stratified to age, gender, chronic disease and infection severity

The median C-reactive protein had a significant relationship with the age groups of the patients who were chosen (p 0.001), with 54 patients aged 45 having the median CRP of 11.30 (IQR: 4.100-27.30) and 68 patients aged > 45 having the median CRP of 29.75. (IQR: 13.38-66.88). In comparison with younger people, elderly adults had higher median values. Gender, however, did not significantly affect CRP median (p = 0.305), with 43 females and 79 males both having a median of 20.10 (IQR: 6.300-53.10). (IQR: 11.30-66.80). Baseline CRP values are affected by factors like age, gender, smoking status, weight, blood pressure, cholesterol levels, and inheritance [(17)]. The CRP prefers to attach to

phosphocholine, which is highly expressed on the surface of damaged cells. These binding changes phagocytic activity to eliminate infections and damaged cells from the body and activates the immune system's classical complement pathway. CRP concentration decreases when the inflammation or tissue damage is healed, giving it a helpful indicator for tracking the severity of disease [(18)].

Correlation of caspase-8 along with WBC count and CRP

the results of the current investigation showed that the CASP-8 levels were significantly increased with the increase of CRP levels ($p < 0.05$) making both of them as a useful prognostic indicator for the development of infection. In the present investigation, there was conversely statistically weak significant relationship that was detected between the caspase-8 levels and the WBC count ($rs = -0.27$; $p < 0.01$). Increasing the level of caspase-8 will decrease the WBC count. The cases were divided into severity groups and a healthy control group to investigate whether the caspase-8 serum level was influenced by any of the characteristics in COVID-19 cases. Receiver-operating characteristic (ROC) analysis is a useful technique for evaluating the efficacy of diagnostic procedures and, more generally, for the precision of a statistical model (e.g., logistic regression). The predictive value of the caspase-8 area under the curve was demonstrated by ROC curve analysis (AUC = 0.88; 95% CI = 0.824-0.936; cut-off value = 1.12 ng/ml; sensitivity = 86.07%; specificity = 87.5%). Results showed that the amount of caspase-8 during COVID-19 infection was a highly accurate biomarker for the progression of infection.

CONCLUSION

The most severe SARS-CoV-2 infections appeared in the wild type and during the emergence of the alpha variant. The median levels of caspase-8 were highest during early infection, when the immune system was still in control of apoptosis, and late infection, when the virus had already spread. The findings confirm that the levels of caspase-8 and CRP during COVID-19 infection were effective biomarkers for the development of infection severity. However, greater study on this topic is necessary.

Financial support and sponsorship: Nil.
conflict of interest: The authors declared no conflict of interest.

Sources of funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

REFERENCES

1. **Zhu N *et al.* (2020):** A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med., 382: 727–733.
2. **Holmes E C *et al.* (2021):** The origins of SARS-CoV-2: a critical review. Cell, 184: 4848–4856.
3. **Dawood A , Dawood Z (2021):** How will the second wave of the dreadful COVID-19 be with the increasing number of the infected cases and mortality in Iraq? Vacunas, 22 (2): 114–118.
4. **Di Giorgio S, Martignano F, Torcia M G *et al.* (2020):** Evidence for host-dependent RNA editing in the transcriptome of SARS-CoV-2. Sci. Adv., 6: eabb5813.
5. **Greaney A *et al.* (2021):** Complete mapping of mutations to the SARS-CoV-2 spike receptor-binding domain that escape antibody recognition. Cell Host Microbe, 29: 44–57.
6. **Galloway S *et al.* (2021):** Emergence of SARS-CoV-2 B.1.1.7 Lineage - United States. doi:10.15585/mmwr.mm7003e2.
7. **Tegally H *et al.* (2021):** Detection of a SARS-CoV-2 variant of concern in South Africa. Nature, 592 (7854): 438-443, doi: 10.1038/s41586-021-03402-9.
8. **Faria N, Mellan T, Whittaker C (2021):** Genomics and epidemiology of a novel SARS-CoV-2 lineage in Manaus, Brazil. DOI: 10.1101/2021.02.26.21252554.
9. **Subramoney K *et al.* (2021):** Identification of SARS-COV-2 omicron variant using spike gene target failure and genotyping assays, Gauteng, south Africa, 2021. Journal of medical virology, *94*(8): 3676-3684. doi: 10.1002/jmv.27797.
10. **Amaral M , Bortoluci K (2020):** Caspase-8 and FADD: where cell death and inflammation collide. Immunity, 52: 890–892.
11. **Nailwal H, Chan F (2019):** Necroptosis in anti-viral inflammation. Cell Death Differ., 26: 4–13.
12. **Bi Q, Wu Y, Mei S *et al.* (2020):** Epidemiology and transmission of COVID-19 in 391 cases and 1286 of their close contacts in Shenzhen, China: a retrospective cohort study. The Lancet Infectious Diseases, 20 (8): 911-919.
13. **Mangalmurti N , Hunter C (2020):** Cytokine storms: understanding COVID-19. Immunity, 53: 19–25.
14. **Baron D , Franchini M, Goobie S *et al.* (2020):** Patient Blood Management During the COVID–19 Pandemic: A Narrative Review. Anaesthesia, 75: 1105–1113. doi: 10.1111/anae.15095.
15. **Pinto B, Oliveira A, Singh Y *et al.* (2020):** ACE2 expression is increased in the lungs of patients with comorbidities associated with severe COVID-19. J Infect Dis., 222:556–563.
16. **Zheng S, Fan J, Yu F *et al.* (2020):** Viral load dynamics and disease severity in patients infected with SARS-CoV-2 in Zhejiang province, China. https://doi.org/10.1136/bmj.m1443.
17. **Chi Y, Ge Y, Wu B *et al.* (2020):** Serum cytokine and chemokine profile in relation to the severity of coronavirus disease 2019 in China. J Infect Dis., 222: 746–754. doi: 10.1093/infdis/jiaa363.
18. **Ali N (2020):** Elevated level of C-reactive protein may be an early marker to predict risk for severity of COVID-19. J Med Virol., 92 (11): 2409-2411. doi: 10.1002/jmv.26097.
19. **Chen J, Wang R, Gilby N *et al.* (2021):** Omicron (B.1.1.529): Infectivity, vaccine breakthrough, and antibody resistance. ArXiv, arXiv., 2112.01318v1.