ISSN: 0067-2904

Exploring the Role of Caspase-3 and IL32 in SARS-CoV-2 Infection among Iraqi Patients

Thaer A. Abed Alhussien, Hula Y. Fadhil *
Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq

Received: 1/8/2022 Accepted: 3/10/2022 Published: 30/8/2023

Abstract

Understanding Caspase-3 (CASP-3) and interleukin-32 (IL32) roles in SARS-CoV-2 infection is critical to linearize the pathogenesis of the virus as well as the resultant disease which may uncover novel therapeutic targets in treating COVID-19 patients. This study aimed to evaluate caspase-3 (CASP3) and interleukin 32 (IL32) roles and their correlation with the disease severity among patients. The case-control study (140 patients and 60 healthy controls) was performed with molecular and ELISA assays. CASP3 and IL32 serum levels were determined along with other clinical data of patients. CASP3 levels were classified as significantly higher ($p < 0.001$), while IL-32 levels were significantly lower in production ($p < 0.001$) in most critical and severe patients compared with mild-moderate. Additionally, IL 32 production reduced with older age, whereas CASP3 levels elevated with older age compared to younger years. Also, the IL-32 showed a protective factor from the severity of the SARS-CoV-2 progression as a negative correlation with the biomarkers (lactate dehydrogenase (LDH), ferritin and D. dimer) values. Thus, IL-32 appeared as the opposite effect of the CASP3 on the severity of COVID-19 infections. ROC curve analysis indicated that IL32 is a good predictor in providing information about the severity of the disease and host recovery. Whereas CASP3 is associated with progressive disease (cut-off value = 160.7 pg/mL; 5.64 ng/mL respectively). CASP3 and IL32 can possibly provide information about the COVID-19 and could be used as cytokine markers to detect SARS-CoV2 infection severity and determine the most reliable treatment plan. More research is needed on this subject.

Keywords: SARS-COV-2; Caspase-3; Interleukin 32; Lactate dehydrogenase; Ferritin; D-dimer.

أستكشاف دور انزيم Caspase-3 و IL32 في عدوى المتلازمة التنفسية الحادة الوخيمة لفيروس كورونا النوع الثاني بين المرضى العراقيين

ثائر عبد الحسين ، حلا يونس فاضل *

قسم البايولوجي، كلية العلوم، جامعة بغداد، بغداد، العراق

الخلاصة

فهم دور انزيم ال caspase -3 والانترلوكين-32 (IL 32) في عدوى المتلازمة التنفسية الحادة الوخيمة

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

لفيروس كورونا النوع الثاني ((SARS−COV−2 له أهمية بالغة لتحديد امراضية الفيروس المسبب، والذي قد يكشف عن أهداف علاجية جديدة في علاج مرضى كوفيد−19. هدفت هذه الدراسة إلى تقييم دور انزيم caspase 3 (CASP3)والإنترلوكين 32 (IL32) وارتباطهما بشدة المتلازمة التنفسية الحادة الوخيمة لفيروس كورونا النوع الثاني SARS−CoV−2 بين المرضى المصابين. جريت الدراسة الحالية على 140 مريضًا و60 شخص صحي كسيطرة باستخدام الاختبارات الجزيئية وتقنيةالممتز المناعي المرتبط بالإنزيم (ELISA) لتحديد مستوى مصل CASP3 و IL32 مع البيانات السريرية الأخرى.

كانت نتائج مستويات CASP−3 أعلى بشكل ملحوظ عند قيمة p اقل من 0,001 ،في حين كانت مستويات IL−32 أقل تركيزا عند قيمة p اقل من 0,001 في معظم الحالات الحرجة والشديدة مقارنة بالحالات الخفيفة والمتوسطة لمرضى كوفيد−19. بالإضافة إلى ذلك، انخفض تركيز IL 32، بينما ارتفعت مستويات انزيم caspase −3 في كبار السن بالمقارنة مع اليافعين. كذالك أظهر IL−32 عاملًا وقائيًا من شدة عدوى المتلازمة التنفسية الحادة الوخيمة لفيروس كورونا النوع الثاني المستفحل حيث له علاقة سلبية مع قيم المؤشرات الحيوية (ferritin، D. dimer، (lactate dehydrogenase (LDH). كما اظهر IL−32 تأثيرا معاكسا لأنزيم ال CASP−3 على شدة عدوى كوفيد−19. أشار تحليل منحنى ROC أن IL32 مؤشر جيد لتوفير معلومات حول شدة المرض واستعادة العائل، بينما ارتبط انزيم CASP3 باستفحال المرض حيث كانت (قيمة القطع = 160.7 بيكوغرام / مل؛ 5.64 نانو غرام / مل، على التوالي). من المحتمل أن يوفر انزيم CASP−3 وIL32 معلومات حول مرض كوفيد−19 ويمكن استخدامهما كمعلمات سايتوكونية للكشف عن شدة عدوى المتلازمة التنفسية الحادة الوخيمة لفيروس كورونا النوع الثاني وتحديد خطة علاج أكثر موثوقية. هناك حاجة إلى مزيد من البحث حول هذا الموضوع.

1- Introduction

Iraq is one of the countries with the highest number of cases of severe acute respiratory syndrome coronavirus (SARS-COV-2) infection. For patients with COVID-19, circulating inflammatory biomarkers representing the immune system and inflammation have been considered as prognostic indicators [1]. Interleukin (IL)-32 is a pro-inflammatory cytokine that plays an important role in viral infection responses. Furthermore, little is understood about how IL-32 is activated in response to viral infection or the pathways that underly its antiviral function. T lymphocytes, natural killer cells, epithelial cells, mast cells, keratinocytes, eosinophils and blood monocytes are the main sources of IL-32. Since IL-32 plays different roles in the induction or inhibition of TNF and other inflammatory cytokines [2, 3], it is here forth important to investigate its role in COVID-19 infection.

The molecular pathways that regulate and execute virus-induced apoptosis are unclear. Caspase-3 is important in the execution of apoptosis induced by the extrinsic and intrinsic apoptosis pathways [4].It has been recently discovered that SARS-CoV-2 ORF3a and ORF7a overexpression activates the extrinsic apoptotic pathway and that it is the strongest activator of the expression of inflammatory cytokines. It is unclear whether caspase-3 is a significant contributor to lymphopenia in patients with severe COVID-19 [5].

Insights into SARS-CoV-2 role, as well as its specific encoded proteins in cell death infection and the role of IL 32, is essential for linearizing the virus's pathogenesis and the resulting disease which may lead to the identification of novel therapeutic targets in treating COVID-19 patients. Currently available data does not provide a clear understanding of the relationship between CASP3 and IL32 in PCR positive COVID-19 patients. Therefore, a preliminary study was performed to evaluate caspase-3 (CASP3) and interleukin 32 (IL32) roles in the pathogenesis of SARS-CoV-2 severity among patients with critical, chronic and dread disease. Our study aim was expanded to include assessment of the relationship of CASP3 and IL32 to age, biomarkers (LDH, D. dimer, and ferritin), Ct value of viral positivity and cases

severity. Serum ferritin, LDH and D-dimer levels were positively correlated with COVID-19 severity. An increased level in this inflammatory biomarker indicates body's stress as an indicator of systemic inflammation. With more stress, such as in severe and critical patient states, the level also rises to an even higher degree. Worsening inflammation demonstrates that lower C_t value (higher viral RNA concentration) is associated with excessive inflammation which is highly likely to be a key factor contributing to the induction of cytokine storm and disease progression [6].

2. Materials and Methods

2.1. Study Population

This case-control study was performed on 200 subjects, including 140 COVID-19 patients and 60 healthy controls (HC). Patients were admitted to Al-Shifa Center in Baghdad medical city during the period from first of December 2020 to the end of April 2021. Practical part of the study was accomplished in Baghdad Central Public Health Laboratory (CPHL) / Molecular Biology Department and Molecular Biology Laboratory at the College of Science, Department of Biology, University of Baghdad. The ages of the patients ranged from 18- >70 years of both sexes. COVID-19 patients were grouped into mild-moderate (46), severe (58), and critical (36) (including 8 cases who died) according to the WHO severity classification. Moreover, all critical and some severe cases were accompanied by dread diseases like renal failure (20), autoimmune (3), hematological disorder and cancer (25). The Ethics Committee of the Iraqi Ministry of Health and Environment and Department of Biology (College of Science, University of Baghdad) (Ref: CSEC/1120/0050) approved the study protocol. Written consent was obtained from all participants for blood collection along with nasopharyngeal swabs.

2.2. Laboratory methods

Nasopharyngeal (NP) swabs, together with 5 ml of blood samples, were collected from the study groups. Samples collected from swabs were directly put into viral transport media (VTM). A total of 200 µl of each NP sample was used for viral RNA extraction via the Exiprep^{TM}Plus Viral DNA/RNA Kit (Bioneer, Korea). SARS-CoV-2 infection detection was performed on swabs samples by specific real-time reverse transcription-polymerase chain reaction (RT-PCR) by *AccuPower*® SARS-COV-2 Multiplex Real-Time RT-PCR Kit, and detecting specific sequences in the E, N and RdRp genes [7]. Blood samples were split into two tubes (sodium citrate and gel tubes). First part of the blood samples (sodium citrate tubes) was centrifuged for 20 minutes at 3500 rpm to obtain plasma which was later used for determining D-Dimer. The second part of the blood samples (*g*el and clot activator tube) was centrifuged for 10 minutes at 6000 rpm to obtain serum for detecting and assessing lactate dehydrogenase (LDH), ferritin, caspase-3 and IL32. Plasma concentration of D-dimer was evaluated by using a specific automated protein analyzer (BIOSENCER STANDARD F200 analyzer) provided by Suwon-si, Gyeonggi Co., Ltd. Korea 2020. Plasma samples for all patients were applied to the instrument and then D-dimer concentration was calculated automatically. LDH serum concentrations were evaluated by using electro-chemiluminescence immunoassay, Roche Cobas Integra 400 plus (Roche Diagnostics GmbH, Mannheim, Germany). Serum samples for all patients were applied to the instrument and then LDH concentration was calculated automatically. Ferritin serum concentration was evaluated by using miniVIDAS analyzer for the fluorescent enzymatic detection, using the technique Enzyme-Linked Fluorescent Assay (ELFA) (BioMerieux). Serum samples for all patients were applied to the instrument for automatic calculation of ferritin concentration. CASP3 and IL32 were determined in serum using an enzyme-linked immunosorbent assay (ELISA) kit manufactured by SunLong Biotech, China (Catalogue Number: RDEEH0546 for CASP3 and RDEEH1938 for IL32), following the manufacturer's instructions. The detection range of the CASP3 assay was 0.313 – 20 ng/ml with

a sensitivity of 0.188 ng/mL while that of IL32 assay was 15.625-1000 Pg/mL with a sensitivity of 9.375 Pg/mL

.

2.3. Statistical analysis

Kruskal-Wallis and Mann-Whitney *U* tests probabilities were used to compare nonparametric variables and to assess significant differences between medians (Asterisk indicates significant differences). They were expressed as the median and interquartile range (IQR). A probability (*p*) value < 0.001 was taken as statistically significant. Spearman's rank-order correlation was performed to analyze the correlation coefficient between caspase-3 and IL-32 along with biomarkers (Ferritin, D. dimer and LDH) in COVID-19 patients. The diagnostic validity of IL32 and CASP3 in distinguishing severe COVID19 cases was assessed by receiver operating characteristic (ROC) curve analysis used to estimate area under the curve (AUC), 95% confidence interval (CI). Youden index, sensitivity and specificity were calculated to define the best cut off values to assess the validity of the significantly different parameters across the disease severity and in predicting severe or critical disease._Logistic regression analysis was applied to calculate odds ratio (OR) and 95% CI under three models: I (unadjusted), II (age-adjusted) and III (age and gender-adjusted). For this analysis, patients and HC were distributed as low and high production groups according to a median of caspase-3 and IL-32 (≤ and > median, respectively), and the high production group was used as the reference category. Statistical package IBM SPSS Statistics 25.0 (Armonk, NY: IBM Corp.) and GraphPad Prism version 8.0.0 (San Diego, California USA) were used to perform statistical analysis. G*Power version 3.1.9.2 software was used to calculate power of sample size (type of power analysis: compromise; α error probability: 0.05; power (1-β error probability): 0.80; effect sized: 0.55; actual power: 0.80).

3. Results

3.1. Main characteristics

The results of CASP3 and IL 32 stratified to baseline characteristics showed significant differences among all patient age groups compared with the control groups, p-value of CASP3 ($p < 0.001$ *vs* p-value = 0.08) and, P -value of IL 32 ($p < 0.001$ *vs* p-value = 0.35) (Table 1). Furthermore, the cross-tabulation of patients' age across CASP3 and IL 32 revealed a statistically significant association between patients' ages with CASP3 and IL 32 levels. CASP3 levels elevated with older age to reach the highest at age ≥ 70 years compared to younger (18-40) years [8.29 IQR (7.87-8-47) *vs*. 4.49 IQR (3.59-5.12)]. Whereas IL32 production reduced with older age to reach the lowest at age ≥ 70 years compared to younger (18-40) years [22.96 IQR (14.90-50.91) *vs*. 238.75 IQR (213.22- 277.98)]. Regarding the gender, it did not show significant differences in CASP3 and IL 32 levels between males and females compared with HC (*p* value = 0.81 *vs*. 0.18 and *p* value = 0.41 *vs*. 0.85) respectively.

Table 1: Median levels of caspase-3 and IL-32 stratified according to characteristics of COVID-19 patients and healthy controls.

Character		Caspase-3 median (IQR); ng/mL Patient	Control	IL-32 median (IQR); pg/mL Patient	Control
Age Group	18-40	4.49 (3.59 - 5.12)	3.8 (3.7 – 4.26)	238.75 (213.22 - 277.98)	2.8 (0.12 – 7.7)
	41-50	6.27 (5.19 -6.85)	3.7 (3.7 – 4.72)	127.23 (101.16 - 219.40)	7.4 (0.4 – 17.86)
	51-60	7.27 (7.09-7.77)	5.2 (5.04 – 5.36)	96.59 (77.25 - 114.87)	1 (0.7 – 0.14)
	61->70	8.29 (7.87-8.47)	--	22.96 (14.90 - 50.91)	--
	p-value	< **0.001**	0.08	< **0.001**	0.35
Sex	Male	6.95 (6.25 - 7.41)	3.98 (3.67 – 4.37)	123.33 (101.16 - 131.53)	2.81 (0.13 – 9.8)
	Female	6.24 (6.04 - 7.13)	3.7 (3.56 – 4.65)	105.73 (87.19 -137.98)	1.47 (0.12 – 17.32)
	p-value	0.81	0.18	0.41	0.85
DM	Yes	7.73 (7.49 - 8.25)	NA	68.65 (26.18 – 95.25)	NA
	No	5.05 (4.21 – 5.37)		219.4 (189.03 – 246)	
	p-value	< **0.001**		< **0.001**	
HTN	Yes	7.89 (7.6 – 8.31)	NA	47.42 (22.7 – 87.19)	NA
	No	5.26 (4.92 – 6)		191.46 (151.68 – 219.67)	
	p-value	< **0.001**		< **0.001**	
Other Disease	No	5.67 (5.2 – 6.27)	NA	154.77 (127.23 – 201.39)	NA
	A	8.4 (7.51 – 8.5)		24.31 (12.75 – 100.35)	
	B	8.34 (8.06 – 8.63)		16.25 (9.8 – 22.7)	
	C	7.76 (7.68 – 8.31)		47.42 (35.59 – 68.65)	
	p-value	< **0.001**		< **0.001**	

IQR: Interquartile range; NA: Not applicable; DM: Diabetic mellites; HTN: Hypertension; A: hematological disorder and cancer; B: renal failure; C: autoimmune; *p*: Kruskal-Wallis test and Mann-Whitney *U* test probability was used to assess significant differences between medians (Asterisk indicates significant differences).

3.2. CASP3 and IL 32 levels across disease severity and several biomarkers

CASP3 and IL-32 (median and interquartile range) in COVID-19 patients according to disease severity, duration of infection, and C_t values detection are shown in Figure 1. CASP3 levels were classified as significantly higher ($p < 0.001$) in most critical and severe cases, including (chronic and dread disease) COVID-19 patients compared with mild-moderate. On the contrary, IL-32 levels were significantly lower production ($p < 0.001$) in most critical and severe patients compared with mild-moderate. Furthermore, higher levels of CASP3 and lower levels of IL32 had significant relationships with RT-PCR Ct values and during the first days of infection ($p < 0.001$).

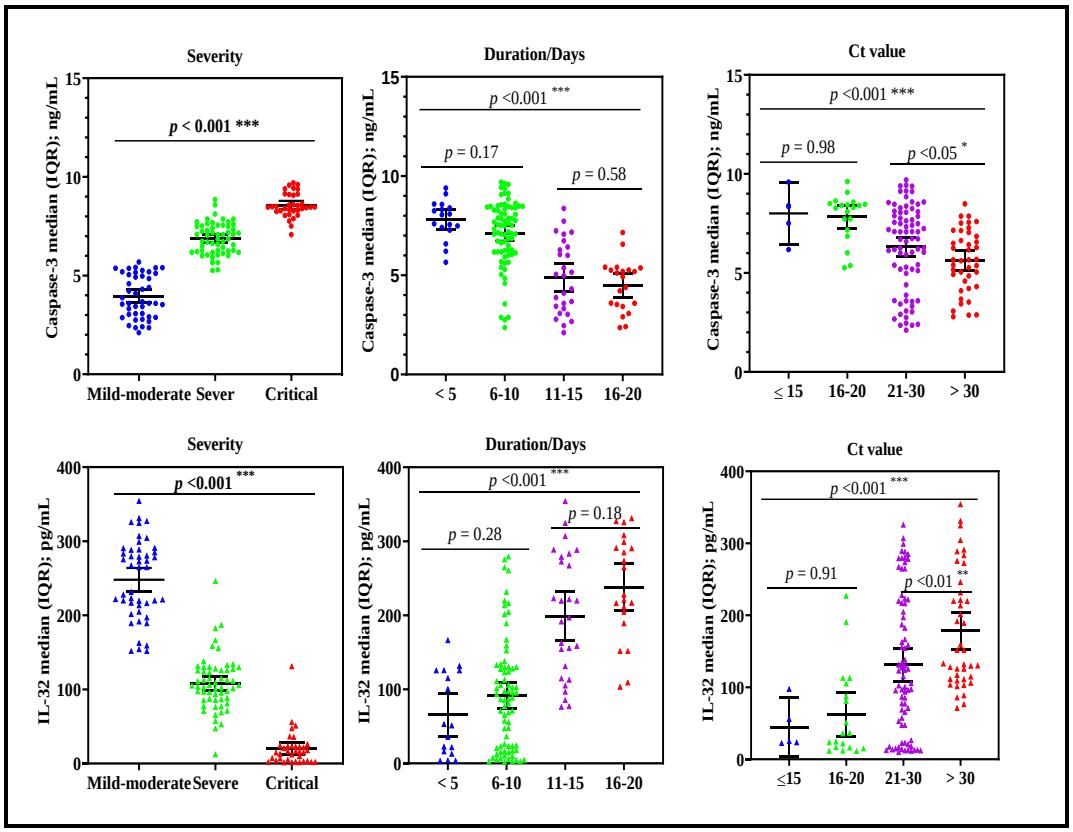

Figure 1: Scatter dot plot of caspase-3 and IL-32 (median and interquartile range) in COVID-19 patients according to disease severity, duration of infection and Ct values detection. Kruskal-Wallis test and Mann-Whitney *U* test was used to assess significant differences between medians (Asterisk indicates significant differences).

Moreover, Spearman's rank-order correlation analysis revealed a significant relationship between CASP3 and IL-32, as well as with biomarkers (ferritin, D. dimer and LDH) as shown in Figure 2. The IL-32 showed a protective factor from the severity of the SARS-CoV-2 progressive as a negative correlation with the value of the biomarker. Thus, IL-32 appeared the opposite effect of the CASP3 on the severity of COVID-19 infections.

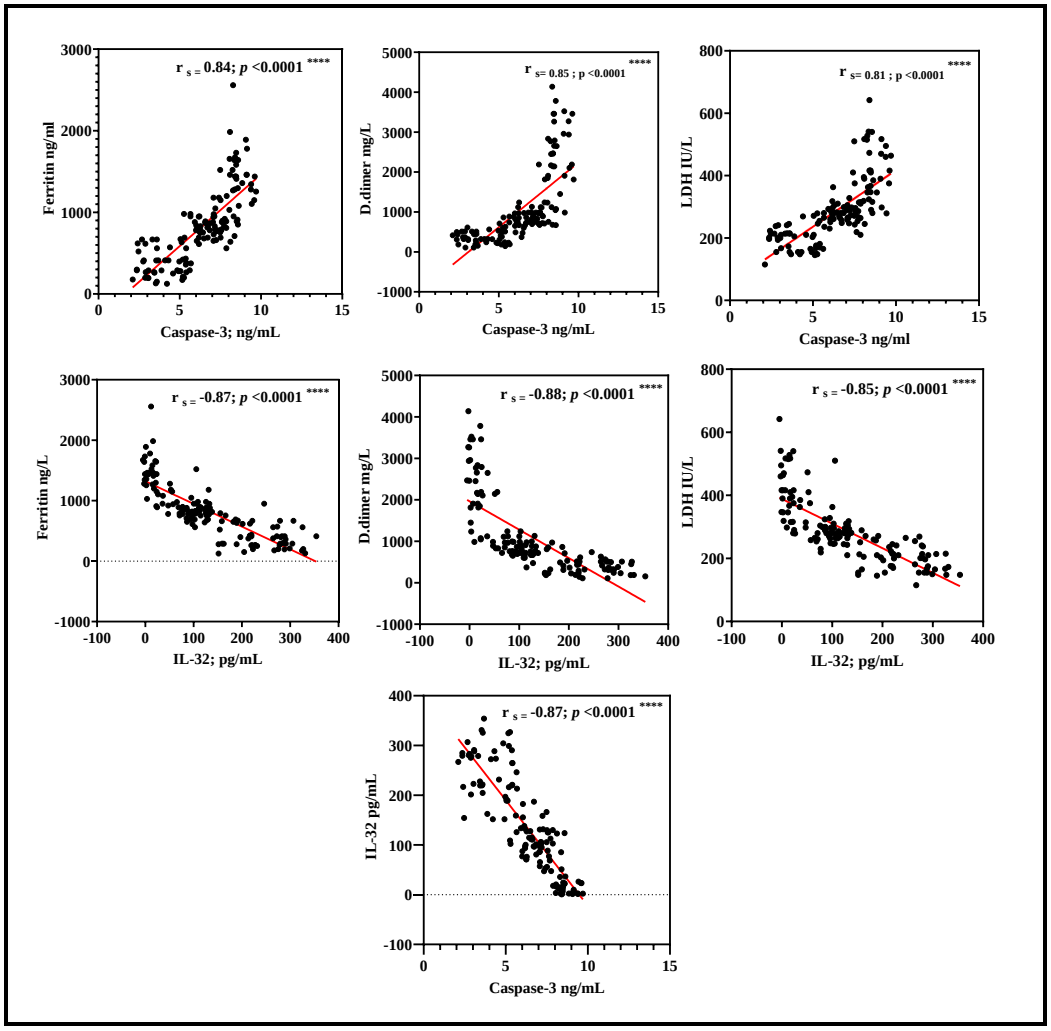

Figure 2: Spearman's rank correlation coefficient (r_s) between caspase-3 and IL-32 along with biomarkers (Ferritin, D. dimer, and LDH) in COVID-19 patients.

Logistic regression analysis revealed that patients with high CASP3 production and low IL32 level were more likely to develop COVID-19 than patients with low CASP3 production and high IL32 level, regardless of age or gender (age and gender adjusted OR = 2.5; 95% CI = 1.55 − 4.02; p = 0.001 *vs*. OR = 1.89; 95% CI = 0.66 − 5.42; p = 0.42) (Table 2).

Table 2: Logistic regression analysis of caspase-3 and IL-32 in COVID-19 patients stratified according to the median of caspase-3 and IL-32 (≤ and > median) in healthy controls.

Model†		OR	95% CI	*p*-value
I (unadjusted)	Caspase-3	2.27	1.71-3.05	< **0.001**
	IL-32	1.08	1.04 – 1.12	< **0.001**
II (age adjusted)	Caspase-3	1.92	1.31-2.82	**0.001**
	IL-32	1.41	0.87-2.29	0.16
III (age and gender adjusted)	Caspase-3	2.5	1.55-4.02	< **0.001**
	IL-32	1.89	0.66-5.42	0.24

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

Receiver operating characteristic curve analysis indicated that CASP3 is a good predictor of severity of disease (area under the curve = 0.996; 95% confidence interval = 0.99 – 1.0; $p < 0.001$; cut-off value = 5.64 ng/mL; sensitivity = 97.9%; specificity = 95.7%). While ROC curve analysis indicated that IL32 is a good predictor providing information about the severity of the disease and host recovery (area under the curve = 0.99; 95% confidence interval = 0.98 – 1.0; $p < 0.001$; cut-off value = 160.7 Pg/mL; sensitivity = 95.7%; specificity = 93.5%) as shown in Figure 3.

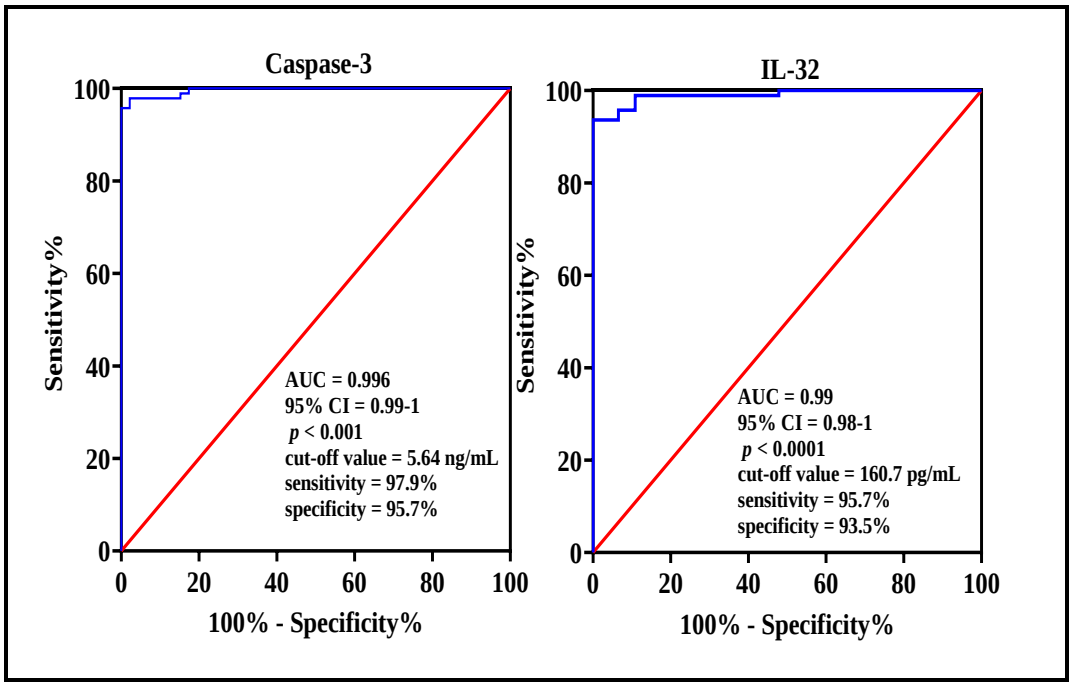

Figure 3: ROC curve analysis of caspase-3 and IL-32 in COVID-19 patients with severe and critical infections *versus* mild infection to predict the recovery of disease (AUC: area under the curve).

2. Discussion

In the current study, the role of CASP3 and IL32 in the pathogenesis of COVID-19 was explored by determining CASP3 and IL32 levels in peripheral blood of COVID-19 patients and their correlation with severity and several prognostic factors (LDH, ferritin and D-dimer), as well as to clarify the correlation of CASP3 and IL32 with aging, gender, progression, duration and cycle threshold (Ct) values of RT-PCR. CASP3 and IL32 concentrations were found to be statistically higher in all patient groups when compared to HC ($p < 0.001$). What do other researchers have to suggest is interesting. Plassmeyer *et al.* noticed that RBCs from acute COVID-19 subjects had increased caspase-3/7 activity when compared to healthy controls [8]. An other study conducted by Hajizadeh *et al.* found the COVID-19 patients have increased caspase-3 expression in their seminal plasma than the healthy controls [9]. COVID-19 induces apoptotic cell death in a diversity of immunological and non-immune cell types, making it a potential therapeutic target [10]. It has recently been discovered that SARS-CoV-2 ORF3a overexpression activates the extrinsic apoptotic pathway, cleaves/activates caspase-9 and increases caspase-3 expression [11]. Deactivated ORF3a reduces viral replication and inhibition cell death caused by SARS-CoV infection [12]. In recent years, IL-32 role has been investigated in a variety of viral infections. Patients with chronic HIV infection, influenza A virus, HCV and HBV, have been found to have the highest IL-32 levels [13].

On the contrary, Bergantini *et al*. observed reduced IL-32 concentration in COVID-19 patients than in the controls [14]. Studies on IL-32 role in COVID-19 infection are still limited. In the current study, we observed that IL32 was significantly higher in patient groups compared to the HC, with significant differences between patient groups.

Studies have reported that as/when ferritin, LDH and D -dimer levels increase the severity of disease also increases [15]. Hyperferritinemia is a marker of severe systemic disease and poor outcomes in COVID-19. It is derived from ferritin which is released by dying, exhausted cells leading to increased iron reacting with oxygen and producing ROS which damages the mitochondrion [16]. Mitochondrial apoptosis is usually triggered by the release of mitochondrial proteins (such as cytochrome c) which leads to caspase-9 and caspase-3 activation [17].

With systemic iron accumulation, there is a rise in cellular oxidative stress, inflammatory cytokine production, a reduction in cellular oxygen consumption, lipid peroxidation and a change from aerobic (pyruvate) to anaerobic (lactate) metabolism via lactic dehydrogenase (LDH) [16]. Inflammatory biomarkers can induce apoptosis of lymphocytes and metabolic disorders like hyperlactatemia can destroy lymphocytes [18]. The increase of ROS causes thrombosis by stimulating the apoptotic caspase cascade. Koupenova *et al*. revealed that there is higher activation of the effector Casp3 in certain patients' platelets and SARS-CoV-2 and caspase-3 colocalized on the same platelet [19]. Increased D-dimer levels may be the consequence of coagulopathy induced by apoptotic endothelial cells. Uncontrolled apoptosis of alveolar endothelial cells can damage oxygenation in a variety of ways, including microvascular apoptosis-induced thrombi in the alveolar vascular bed and vascular fluid leaks into lung tissue. Platelets apoptosis as a result of the mitochondrial disruption, inducing them to clot $\geq$ 50 times faster than normal platelets and enhancing the production of thrombi in COVID-19. This results in metabolic dysregulation triggering the coagulation cascade, thus causing D-dimer increases [20].

In our study, we found conversely, statistically significant inverse relationship between IL32 levels and ferritin $r_s = 0.87$; $p < 0.0001$, between IL32 levels and LDH $r_s = 0.85$; $p < 0.0001$, and between IL32 levels and D-dimer $r_s = 0.88$; $p < 0.0001$. IL32 decreases irrespective of disease severity. Rasool *et al*. observed that increased levels of IL-32 during HIV infection may block the viral replication [21]. IL-32 may assist the neutralization of viral components (e.g., through the deactivation of viral nucleocapsid structures). IL-32 may help to reduce both systemic and local inflammation, which may be effective in mild-moderate COVID-19 patients but is more likely to fail to be effective in severe patients [22].

In our findings we noticed that CAPS3 was negatively significant with Ct value ($p < 0.001$), while IL32 was positively significant with Ct value ($p < 0.001$) and the first days of infection played a significant role ($p < 0.001$) in CASP3 and IL32 levels. The rate of apoptosis is regulated by viral RNA replication and the period of the diseases [23]. The high viral load was significantly correlated with levels of IFN, TNF and tumor necrosis factor-related apoptosis-inducing ligand. Truncated ORF3b of SARS CoV2 suppresses IFN induction more effectively than SARS-CoV which could explain the poor IFN response found in COVID-19 patients [24]. In addition, IL-32 and IFN have a positive relationship, and IL32 deficiency has been associated to a decrease in Th1 cells and IFN [25].

Conclusion

CASP3 and IL32 could be used as biomarkers to detect the severity of SARS-CoV2 infection-induced cell damage and can also determine the most reliable treatment plan. Anti-CASP-3 drugs and up-regulated IL32 may indeed be useful for treating COVID-19 patients. To fully understand the latent potential and characteristics of the current topic, additional studies on the activity level of the CASP3 and IL32 are required.

Funding

This study received no particular support from governmental, private or non-profit funding bodies.

Conflict of Interest

There were no conflicts of interest declared by the authors.

Acknowledgments

The authors thank the medical staff at Al-Shifa Medical Center at Baghdad Medical City, as well as the Central Public Health Laboratory (CPHL) / Molecular biology department in Baghdad for their kind cooperation.

References

[1] Z. Mahmood *et al.*, "Severity of coronavirus disease 19: Profile of inflammatory markers and *ACE* (rs4646994) and *ACE2* (rs2285666) gene polymorphisms in Iraqi patients." *Meta gene*, vol. 31, pp. 1-6, 2022. 101014. Available: doi: 10.1016/j.mgene.2022.101014

[2] S. Han and Y. Young. "Interleukin-32: Frenemy in cancer?" *BMB reports*, vol. 52, no. 3, pp. 165-174, 2019. Available: doi:10.5483/BMBRep.2019.52.3.019

[3] H. Jeong et al. "Interleukin-32-induced thymic stromal lymphopoietin plays a critical role in macrophage differentiation through the activation of caspase-1 in vitro." *Arthritis research & therapy* vol. 14, no. 6, pp. R259. 28 Nov. 2012, doi:10.1186/ar4104

[4] D. Tang *et al.*, "The molecular machinery of regulated cell death." *Cell research*, vol. 29, no. 5, pp. 347-364, 2019. Available: doi:10.1038/s41422-019-0164-5

[5] F. Sing and D. Liu. "Similarities and Dissimilarities of COVID-19 and Other Coronavirus Diseases." *Annual review of microbiology*, vol. 75, pp. 19-47, 2021. Available: doi:10.1146 /annurev-micro-110520-023212

[6] A. Rabaan, *et al*. "Viral Dynamics and Real-Time RT-PCR Ct Values Correlation with Disease Severity in COVID-19." *Diagnostics (Basel, Switzerland)*, vol. 11, no. 6, pp. 1091. 15 Jun. 2021. Available: doi:10.3390/diagnostics11061091

[7] Z. Mahmood *et al.*, "Estimation of Hematological Parameters of Disease Severity in Iraqi Patients with COVID-19". *Iraqi Journal of Science*, vol. 62, no. 10, pp. 3487-3496, 2021. Available: 10.24996/ijs.2021.62.10.8.

[8] M. Plassmeyer *et al.*, "Caspases and therapeutic potential of caspase inhibitors in moderate-severe SARS-CoV-2 infection and long COVID." *Allergy*, vol. 77, no. 1, pp. 118-129, 2022. Available: doi:10.1111/all.14907

[9] H. Behzad, and B. Tartibian. "COVID-19 and male reproductive function: a prospective, longitudinal cohort study." *Reproduction*, vol. 161, no. 3, pp. 319-331, 2021. Available: doi: 10.1530/REP-20-0382

[10] S. Bader *et al.*, "Programmed cell death: the pathways to severe COVID-19?" *The Biochemical journal*, vol. 479, no. 5, pp. 609-628, 2022. Available: doi:10.1042/BCJ20210602

[11] Y. Ren *et al.*, "The ORF3a protein of SARS-CoV-2 induces apoptosis in cells." *Cellular & molecular immunology*, vol. 17, no. 8, pp. 881-883, 2020. Available: doi:10.1038/s41423-020-0485-9

[12] M. Bianchi *et al.*, "SARS-Cov-2 ORF3a: Mutability and function." *International journal of biological macromolecules*, 170, 820-826, 2021. Available: doi: 10.1016/j.ijbiomac.2020.12.142

[13] F. Ribeiro-Dias *et al.*, "Interleukin 32: a novel player in the control of infectious diseases." *Journal*

of leukocyte biology, vol. 101, no. 1, pp. 39-52, 2017. Available: doi:10.1189/ jlb.4RU0416-175RR

[14] L. Bergantini *et al.*, "Cytokine profiles in the detection of severe lung involvement in hospitalized patients with COVID-19: The IL-8/IL-32 axis." *Cytokine*, vol. 151, pp. 155804, 2022. Available: doi: 10.1016/j.cyto.2022.155804

[15] M. Ozen *et al.*, "D-Dimer as a potential biomarker for disease severity in COVID-19." *The American journal of emergency medicine*, vol. 40, pp. 55-59, 2021. Available: doi: 10.1016/j. ajem.2020.12.023

[16] L. Gibellini, and M. Loredana. "Programmed Cell Death in Health and Disease." *Cells*, vol. 10, no. 7, pp. 1765, 2021. Available: doi:10.3390/cells10071765

[17] J. Liu *et al.*, "Signaling pathways and defense mechanisms of ferroptosis." *The FEBS journal*, vol. 10, no. 6, 2021. Available: doi:10.1111/febs.16059

[18] B. Rajyalakshmi et al., "Prognostic Value of "Cycle Threshold" in Confirmed COVID-19 Patients." *Indian journal of critical care medicine*, vol. 25, no. 3, pp. 322-326, 2021. Available: doi:10.5005/jp-journals-10071-23765

[19] M. Koupenova *et al.*, "SARS-CoV-2 Initiates Programmed Cell Death in Platelets." *Circulation research*, vol. 129, no. 6, pp. 631-646, 2021. Available: doi:10.1161/CIRCRESAHA.121.319117

[20] A. González, "Programmed Cell Death. Review and Its Impact in Covid-19". *Clinical Research and Trials*, vol. 7, pp. 1-11, 2020. Available: doi: 10.15761/CRT.1000330.

[21] S. Rasool *et al.*, "Increased level of IL-32 during human immunodeficiency virus infection suppresses HIV replication." *Immunology letters*, vol. 117, no. 2, pp. 161-7, 2008. Available: doi: 10.1016/j.imlet.2008.01.007

[22] C. Law *et al.*, "Clinical Implications of IL-32, IL-34 and IL-37 in Atherosclerosis: Speculative Role in Cardiovascular Manifestations of COVID-19." *Frontiers in cardiovascular medicine*, vol. 8, no. 6, 2021. Available: doi:10.3389/fcvm.2021.630767

[23] H. Li *et al.*, "SARS-CoV-2 and viral sepsis: observations and hypotheses." *Lancet (London, England)*, vol. 395, no. 10235, pp. 1517-1520, 2020. Available: doi:10.1016/S0140-6736(20)30920-X

[24] Y. Konno *et al.*, "SARS-CoV-2 ORF3b Is a Potent Interferon Antagonist Whose Activity Is Increased by a Naturally Occurring Elongation Variant." *Cell reports*, vol. 32, no. 12, 108185, 2020. Available: doi: 10.1016/j.celrep.2020.108185

[25] L. Han *et al.*, "Interleukin 32 Promotes Foxp3$^+$ Treg Cell Development and CD8$^+$ T Cell Function in Human Esophageal Squamous Cell Carcinoma Microenvironment." *Frontiers in cell and developmental biology*, vol. 9, no. 3, 2021. Available: doi:10.3389/fcell.2021.704853