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Markers of inflammasome activation in Coronavirus disease 2019 (COVID-19) Egyptian patients: Impact on disease severity

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Abstract

There are conflicting data regarding the relationship between coronavirus disease 2019 (COVID-19) severity and Caspase-1 (Casp-1), interleukin-1ß (IL-1β), and IL-18. Our study sought to quantify the levels of IL-18, IL-1ß, and Casp-1 as indicators for inflammasome activation in COVID-19 patients at Assiut University Hospitals and to correlate their levels with parameters of disease severity in COVID-19 patients. Serum levels of Casp-1, IL-1β and IL-18 were measured in 63 COVID-19 patients and 26 normal controls by an enzyme linked immunosorbent assay (ELISA). Also, arterial blood gas analysis and laboratory parameters including hemoglobin, platelets, lymphocyte count, liver function test, kidney function test, C-reactive protein (CRP), D-dimer, ferritin and LDH were estimated. Serum levels of Casp-1, IL-1β and IL-18 were significantly higher in the COVID-19 group as compared to controls (p= 0.04, p=0.001 and p=0.03, respectively). Although the three markers were higher in the severe group, yet only IL-1ß showed a significant difference as compared to the non-severe group (p=0.04). IL-18 had significant positive correlations with CRP and ferritin (p = 0.04 and p = 0.02, respectively). IL-1β was positively correlated with alanine aminotransferase. Casp-1 had significant positive correlations with CRP and lactate dehydrogenase (p=0.045 and p=0.001, respectively). Patients showed weak positive correlations between serum level of Casp-1 and each of IL-1β and IL-18. Also, a strong positive correlation was found between IL-1β and IL-18 ($p < 0.0001$). In conclusion, inflammasome activation was a hallmark in COVID-19 patients. The markers of activation were positively correlated with many parameters of inflammation, may suggest their important roles in the pathophysiology of the disease and its progression. IL-1β was the only marker to be correlated with disease severity and therefore may be suggested as a potential marker for identifying severe COVID-19 patients.

Keywords: Inflammasome; COVID-19, Casp-1, IL-1β, IL-18.

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Introduction

Coronavirus disease 2019 (COVID-19) is a serious respiratory infection characterized by fever, dry cough, and dyspnea.[1] A significant portion of hospitalized patients developed severe hypoxemia and needed ICU-assisted ventilation, even though the majority of infected people only exhibit minor symptoms and do not need to be hospitalized.[2]

Important pathogenic characteristics of severe COVID-19 include hyperinflammation and a cytokine storm.[3,6] Acute respiratory distress and respiratory failure can result from the overactivation of inflammasome signaling, which triggers a massive release of proinflammatory cytokines and amplifies the inflammatory response.[4,7,8]

The inflammasomes are important components that induce inflammation in the innate immune responses. They are large cytoplasmic multiprotein complexes that were first identified as regulators of caspase-1 (Casp-1) activation.[9] They are made up of three fundamental parts: a sensor, the adaptor protein apoptosis-associated speck-like protein having a caspase-recruitment domain (ASC), and the Casp-1. The sensor proteins that make up inflammasomes include NOD-like receptor proteins (NLRPs) 1,2,3 and 4, and AIM-2- like receptor (ALRs). NLRP3 has received the most attention among these inflammasomes. [10]

Interleukin-1ß (IL-1ß) and/or interleukin-18 (IL-18) are proteolytically activated because of caspase activation, which provokes an inflammatory response. Particularly, IL-1ß is regarded as a gatekeeper cytokine that is essential for many processes connected to the induction and control of inflammation.[11]

Inflammasomes have recently been recognized as crucial prognostic indicators of poor COVID-19 outcomes in patients. [12] The NLRP3 inflammasomes are activated in the pulmonary macrophages and blood monocytes, leading to the production of IL-1ß and pyroptosis. Casp-1 activity and IL-18 in the sera were found to be correlated with the severity of COVID-19 disease.[12] Our study aimed to determine whether levels of IL-18, IL-1β, and Casp-1 could be indicators for inflammasome activation in COVID-19 patients, as well as indicators for COVID-19 disease severity.

Subjects and Methods

The present case-control study included 63 patients with confirmed COVID-19 as determined by reverse-transcription polymerase chain reaction (RT-PCR). They were recruited from the isolation and intensive care units of the Chest Department, Assiut University Hospitals. In addition, 20 age- and sex- matched normal controls were also included in the study. The study protocol was reviewed and approved for research ethics by the Institutional Review Board of the Faculty of Medicine, Assiut University (Dated November 2021). All participants provided written informed consent before included in the study.

All study cases were subjected to history taking, clinical examinations, and chest computed tomography (CT). Arterial blood gases [oxygen saturation (SpO_2) and partial pressure of carbon dioxide ($PaCO_2$)] were determined using an automated analyzer (Model 850, Chiron Diagnostics, Medfield, MA). Patients were categorized according to the quick COVID-19 severity index.[13] The nasal cannula flow rate, minimum documented pulse oximetry, and respiratory rate were the three bedside variables used to create the 12-point quick COVID-19 severity index (QCSI). Then, the patients were categorized into three risk groups based on their scores (low risk 0-4, intermediate risk 4-6, and high risk ≥7).[13]

Blood samples were collected from study subjects. For each participant, the lymphocyte count was determined using an automated hematology analyzer (Serial number: 513554, CELL-DYN Ruby 1700, USA). Serum samples were isolated and used for liver function tests, kidney function tests and lactate dehydrogenase (LDH) tests, estimated using a blood chemistry analyzer (Serial number:500558, Cobas integra 400 plus, Switzerland). The ferritin test was measured using an immunoassay analyzer (Serial number: 510552, Beckman Coulter Access 2, USA). The D dimer was measured using the coagulation

analyzer (Serial number: BG8503252, Diagnostica Stago STA Satellite, USA) and the C-reactive protein (CRP) was estimated by CRP-Latex (lot CR350B, Spinreact, Spain). Finally, IL-18, IL-1β, and Casp-1were measured by enzyme-linked immunosorbent assay (ELISA) kits (SinoGeneclon Co., Ltd., Hangzhou, China). The kits are based on the sandwich-ELISA technique. The kits catalogue numbers were SG-10260, SG-10281, and SG-10395 for quantitative assessment of IL-1β, IL-18, and Casp-1, respectively. All previously mentioned methods were carried out according to the manufacturer's instructions.

Statistical analysis

Data analysis was done using Statistical Package for the Social Sciences, version 20 (SPSS Inc., Chicago, IL, USA). The quantitative variables were described in in terms of mean and standard error, while the qualitative variables were described in terms of “numbers" and "percent". The student’s t test was used to compare quantitative parametric variables between two groups. To compare qualitative variables, the chi-square (X^2) test or Fisher's exact test (if the frequency was 5) were used. We evaluated the relationship between two normally distributed variables using Pearson correlation coefficients. A p value <0.05 was considered significant.

Results

Demographic and laboratory data of COVID-19 patients and healthy controls

The mean age of patients was 62.8 ± 15 years and 47.6% of them were males. As shown in Table 1, the lymphocyte count decreased significantly (p= 0.005), while a significant increase was observed in direct bilirubin (p<0.001), ALT (p <0.001), AST (p <0.001) urea (p <0.001), creatinine (p<0.001), ferritin (p<0.001), CRP (p <0.001), and LDH (p<0.001).

Table 1. Demographic and laboratory data of COVID-19 patients and healthy controls.

Variables	Patients (n=63)	Controls (n=26)	*p*-value
Age (years)	62.8 ± 15	57.89±8	NS
Sex#			
Male	30 (47.6%)	15(57.7%)	NS
Female	33 (52.4%)	11(42.3%)	
Hematological parameters			
Lymphocytes (x10^9/L)	1.8±1	3±0.2	0.005
Platelets (x10^9/L)	262.7±15	430±161	<0.001*
Hb (g/dl)	12.2±0.4	13.5±11.5	0.005
Liver function tests			
Direct bilirubin (mg/dl)	9.2±1	0.2±0.05	<0.001*
Albumin (mg/dl)	20±3	41.7±1	<0.001*
ALT (IU/L)	139.9±4	33±3	<0.001*
AST (IU/L)	130.7±10	24±4	<0.001*
Renal function tests			
Urea (mg/dl)	17.9±5	4±0.2	<0.001*
Creatinine ((µmol /dl)	125.3±15	78.6±4	<0.001*
COVID-related biomarkers			
Ferritin (ng/ml)	854.4±143	77.8±6	<0.001*
CRP (mg/dl)	91.4±9.8	0.2 ±0.03	<0.001*
D-dimer (µg/ml)	2.9±0.3	0.4±0.03	<0.001*
LDH (U/L)	602±47.5	146.5±21	<0.001*

Hb: hemoglobin, ALT: alanine aminotransferase, AST: aspartate aminotransferase, CRP: C-reactive protein, LDH: lactate dehydrogenase. Data are presented as Mean ± SD or # number (%),P > 0.05 is not significant (NS).

Demographic, clinical, and laboratory characteristics in severe and non-severe COVID-19 patients

Of the 63 COVID-19 patients, 36 (57.14%) patients were classified as severe cases according to the Quick Severity Index. There were statistically significant differences regarding the pulse oximetry, O2 flow rate, respiratory rate, and ultimately the QCSI between the severe and non-severe patient groups. PaO_2, SO_2 and lymphocyte count were significantly lower in severe than non-severe cases, as shown in Tables 2 and 3. No differences were observed between the two groups regarding the other clinical and laboratory findings.

Table 2. Demographic and clinical data of severe and non-severe CVOID-19 patients.

Variables	Severe (n= 36)	Non-severe (n= 27)	*p*-value
Age (years)	64.4 ± 3	60 ± 3	NS
Sex#			
Male	19 (52.7%)	12 (44.4%)	NS
Female	17 (47.2%)	15 (55.5%)	
Quick Severity Index			
Pulse oximetry	78.54 ± 2.45	90.92 ± 1.53	<0.0001*
O_2 flow rate: (L/min)	6.00 ± 0.18	6.00 ± 0.18	<0.0001*
RR (breaths/min)	34.66 ± 1.84	25.58 ± 1.08	<0.0001*
QCSI	10.23 ± 0.30	4.88 ± 0.31	<0.0001*
Symptoms			
Cough	13 (37%)	9 (34.6%)	NS
Expectoration	24 (68.6%)	17 (65.4%)	NS
Dyspnea	13 (37%)	13 (50%)	NS
Diarrhea	24 (68.6%)	18 (69.2%)	NS
Headache	15 (42.9%)	15 (57.7%)	NS
Myalgia	21 (60%)	14 (53.8%)	NS
Fatigue	7 (20.0%)	6 (23%)	NS
Sore throat	14 (40%)	17 (65.4%)	NS
Anorexia	18 (51.4%)	17 (65.4%)	NS
Fever	8 (30.8%)	6 (17%)	NS
Blood gases			
SO_2 (%)	77.17 ± 2.65	90.73 ± 1.52	<0.0001*
PaO_2 (mmHg)	51.97 ± 2.92	67.65 ± 3.37	0.001*
$PaCO_2$ (mmHg)	38.91 ± 2.38	36.54 ± 2.68	NS
Comorbidity: No. (%)			
HTN	11 (31.4%)	9 (34.6%)	NS
DM	12 (34.3%)	6 (23.1%)	NS
KD	2 (5.7%)	0 (0.0%)	NS
COPD	5 (14.3%)	0 (0.0%)	NS
Others	6 (17.1%)	2 (7.7%)	NS

RR: respiratory rate, QCSI: quick COVID-19 severity index, SO_2: oxygen saturation, PaO_2: partial pressure of oxygen, $PaCO_2$: partial pressure of carbon dioxide, HTN: hypertension, DM: diabetes mellitus, COPD: chronic obstructive pulmonary disease, CKD: chronic kidney disease, others include rheumatoid arthritis, pulmonary embolism, viral pneumonia, hypothyroidism. Data are presented as Mean ± SE, $P > 0.05$ is not significant (NS).

Table 3. Laboratory data of severe and non-severe CVOID-19 patients.

Variables	Severe (n= 36)	Non-severe (n= 27)	*p*-value
Hematological parameters			
Lymphocytes (x10^9/L)	0.8±0.2	2.3±0.2	0.003*
Platelets (x10^9/L)	249.9 ± 15	262.7 ± 35	NS
Hb (g/dl)	12.2 ± 0.4	12 ± 0.4	NS
Liver function tests			
Direct bilirubin (mg/dl)	9.2 ± 1	8 ± 2	NS
Albumin (mg/dl)	18.2± 3	20± 3	NS
ALT (IU/L)	139.9 ± 75	34.4 ± 4	NS
AST (IU/L)	130.7 ± 74	45.9 ± 10	NS
Renal function tests			
Urea (mg/dl)	16.9 ± 2	17.7 ± 5	NS
Creatinine (µmol /dl)	125.3 ± 15	117.8 ± 31	NS
COVID-related biomarkers			
Ferritin (ng/ml)	854.4 ± 267	798.6 ± 143	NS
CRP (mg/dl)	91.4 ± 10	66.4 ± 10	NS
D-dimer (µg/ml)	2.9 ± 0.4	1.7 ± 0.3	NS
LDH (U/L)	602 ± 78	487.5 ± 48	NS

Hb: hemoglobin, ALT: alanine aminotransferase, AST: aspartate aminotransferase, CRP: C-reactive protein, LDH: lactate dehydrogenase. $P > 0.05$ is not significant (NS).

Inflammasome activation in COVID-19 patients and healthy controls

Regarding the inflammasome activation markers Casp-1, IL-1β, and IL-18, COVID-19 patients had significantly higher serum levels compared to the normal controls (10.3 ± 3 vs. 5 ± 0.3 pg/ml, *p*= 0.03, 3.2 ± 0.7 vs. 1 ± 0.2 pg/ml, *p* = 0.001 and 3.5 ± 0.3 vs. 2.6 ± 0.2 pg/ml, *p* = 0.04, respectively), (Figure 1A). Although all three markers were higher in the severe group, only IL-1ß showed a statistical difference (3.7 ± 1 vs. 2.3 ± 0.8 pg/ml, *p*=0.04; as shown in Figure 1B.

Correlations of the markers of inflammasome activation with the clinical and laboratory data in COVID-19 patients

Among different laboratory data, IL-18 had significant positive correlations with CRP and ferritin (r = 0.3, *p* = 0.04 and r=0.3, *p* = 0.02, respectively). In addition, IL-1β was positively correlated with ALT level (r = 0.4, *p* = 0.001). Also, Casp-1 had significant positive correlations with CRP and LDH (r=0.3, *p*=0.045 and r=0.3, *p*=0.001, respectively), Figure 2. However, there was no correlation between the three markers (Casp-1, IL-1β, and IL-18) and clinical findings, including temperature, SO_2, PaO_2, $PaCO_2$, pulse oximetry, O_2 flow rate, respiratory rate, and QCSI.

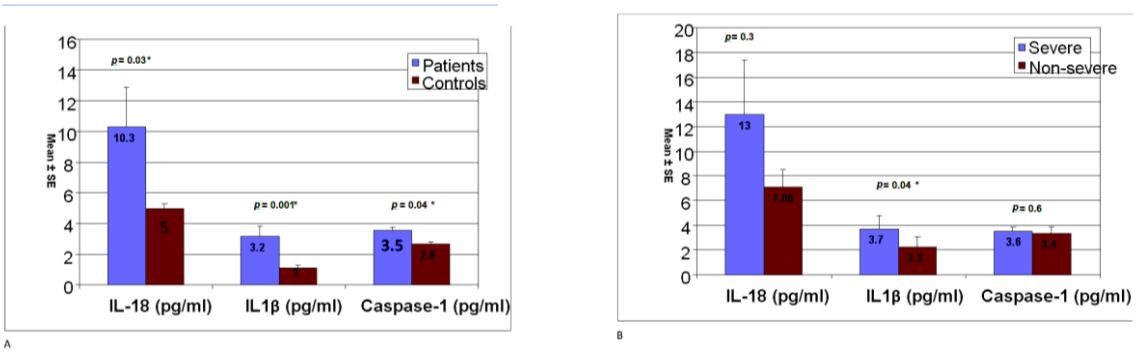

Figure 1. Inflammasome activation markers (A) in COVID-19 patients and controls and (B) between severe and non-severe COVID-19 cases.

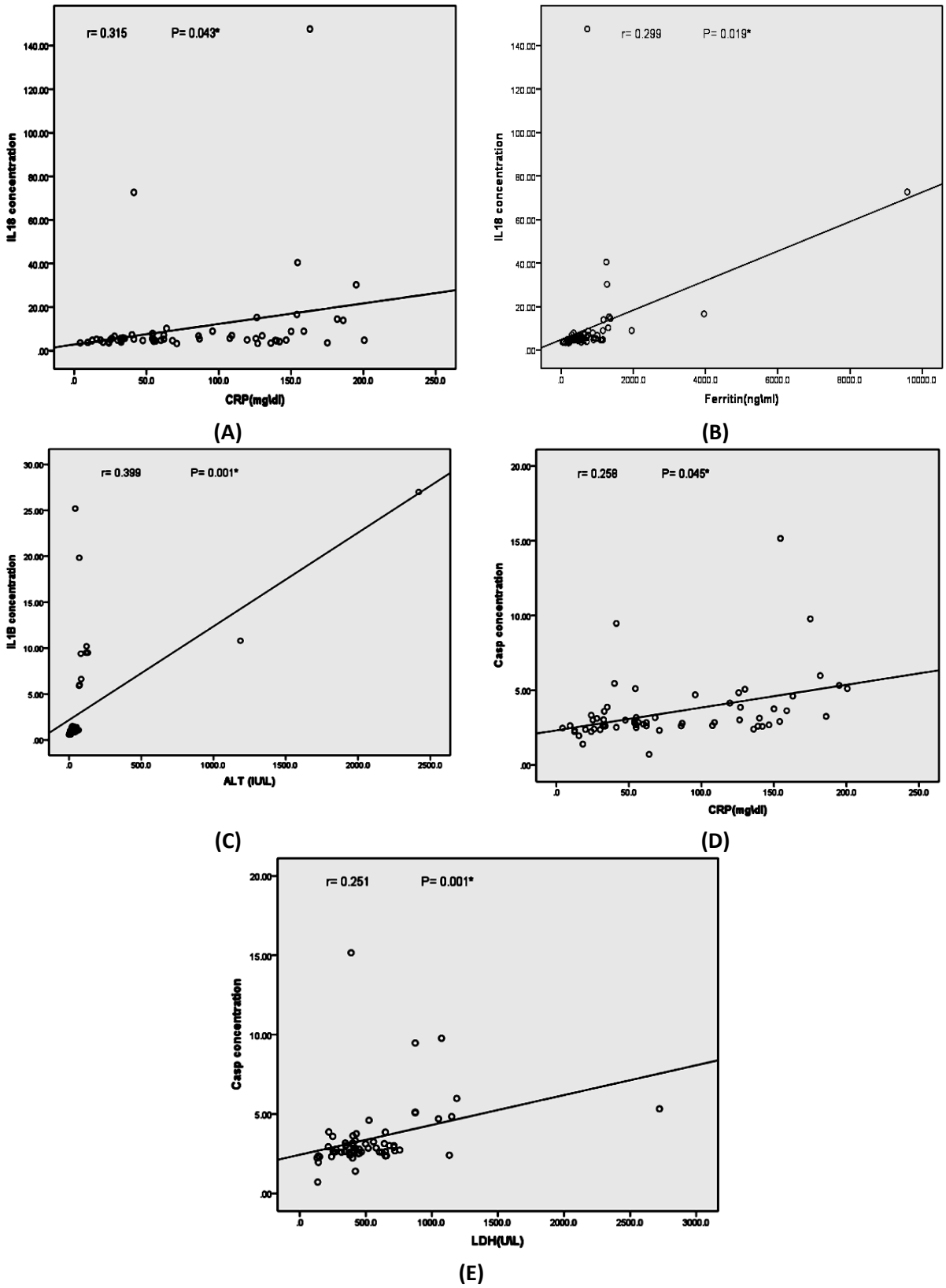

Figure 2. Correlations between markers of inflammasome activation and laboratory findings in COVID-19 patients. (A) between IL-18 and CRP, (B) between IL-18 and ferritin, (C) between IL-1β and ALT level, (D) between Casp-1 and CRP; and (E) between Casp-1 and LDH.

Correlations among markers of inflammasome activation in COVID-Patients

As presented in Figure 3, weak positive correlations were found between the serum level of Casp-1 and each of IL-1β and IL-18 (r = 0.3, p = 0.02 and r = 0.4, p = 0.001, respectively). However, a strong positive correlation was found between IL-1β and IL-18 (r = 0.7, p < 0.0001).

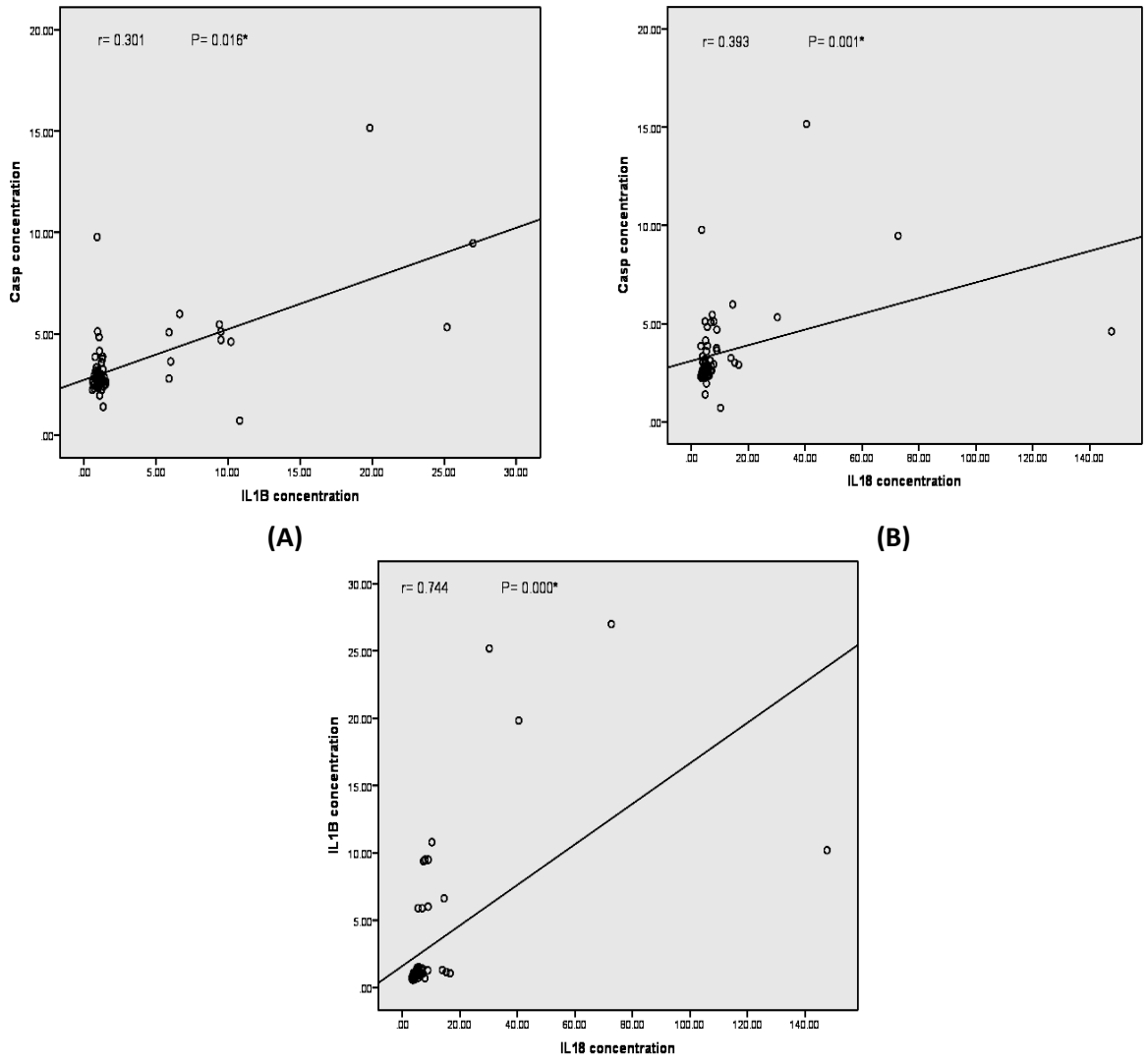

Figure 3. Correlations between serum levels of (A) Casp-1 and IL-1β, (B) Casp-1 and IL-18 and (C) IL-1β and IL-18.

Discussion

In order to identify severe patients and differentiate them from non-severe patients, there is a crucial necessity to identify clinical laboratory predictors of COVID-19 progression toward the severe form.[14] An intense inflammatory response is characteristic of severe COVID-19 infections and may eventually result in organ failure and patient death.[12] Therefore, we aimed to determine whether levels of IL-18, IL-1β, and Casp-1 could be indicators for inflammasome activation in COVID-19 patients, as well as indicators for COVID-19 disease severity.

In our study, we classified patients depending on quick severity index into severe and non-severe groups.[13] We assessed the levels of IL-18, IL-1β, and Casp-1 by ELISA as indicators for inflammasome activation in 63 COVID-19 patients in the chest isolation and intensive care units of Assiut University Hospitals and correlated their levels with markers of disease severity in COVID-19 patients.

In accordance with earlier studies[14, 15], the COVID-19 markers of inflammation and cell injury, including CRP, LDH, D-dimer, and ferritin, were significantly increased in our patients, along with the impairment of liver and kidney functions. Furthermore, *Chopra et al.,* 2020 demonstrated that COVID-19 patients in the intensive care unit had elevated levels of inflammatory markers ferritin, D-dimer, CRP, and LDH.[16]

Proinflammatory cytokines like tumor necrosis factor (TNF) and IL-6 were found to be abundant throughout the disease process.[17] Serum markers of inflammation such as IL-6-inducible hepatic factors CRP and ferritin, as well as the corresponding increase in the concentration of D-dimer, were all present in conjunction with this proinflammatory cytokine response and all linked to a poor prognosis.[18]

Acute-phase IL-1ß can strongly induce this proinflammatory cytokine response, which was also consistent with its known role in IL-6 production.[19] Inflammasome-derived products such as active Casp-1 (Casp1p20) and IL-18 in the sera were also correlated with the markers of COVID-19 severity, including IL-6 and LDH.[12]

Our results showed significantly higher serum levels of Casp-1, IL-1β and IL-18 in COVID-19 patients compared to normal controls. The inflammasomes activate intracellular caspase-1, which cleaves the inactive precursors of IL-1ß and IL-18 into bioactive cytokines in addition to the lytic form of cell death recognized as pyroptosis. [29] Thus, a positive correlation was found in this study between the inflammasome-related biomarkers, pointing to the important role they collectively play in inflammation in these patients, but there were no correlations with the clinical data.

In the present work, although all three markers were higher in the severe group, only IL-1β showed a statistical difference. Also, the three markers did not show any significant correlations with the clinical findings, including temperature, SO2, PaO2, PaCO2, pulse oximetry, O2 flow rate, respiratory rate, and QCSI. However, IL-18 had significant positive correlations with CRP and ferritin, IL-1β was positively correlated with ALT and Casp-1 had significant positive correlations with CRP and LDH. In accordance with these findings, many studies reported the association of different inflammasome related biomarkers with different laboratory findings. Adamik *et al*., 2022 reported that there was a correlation between IL-18 and ferritin levels.[22] Also, Rodrigues *et al.,* 2021 reported the association of active caspase-1, and IL-18 with ferritin, LDH and CRP.[12]

IL-1β was the only marker to be significantly higher among our severe COVID-19 patients. The presence of a significant number of comorbidities in our patients (hypertension, diabetes, kidney diseases, etc.) may contribute to the increased basal IL-1β production, which may be significantly elevated in severe COVID-19 patients.

In a previous study conducted on more than 1500 COVID-19 patients, TNF, IL-6, IL-8, and to a lesser extent, IL-1ß were found to be elevated and their levels correlated with disease outcome and mortality.[17] Also, patients with severe COVID-19 showed markedly elevated levels of TNF-α, IL-1ß and IL-6 as compared to those with moderate disease.[23,24] However, serum IL-1ß and IL-1RA concentrations have been equivocal in many studies. In one study, IL-1ß was found to be markedly increased in patients compared to controls.[1]

Findings relating the inflammasome activation markers to COVID-19 severity are also inconsistent. Chen *et al., 2021* found that the levels of IL-1β and IL-18 were significantly higher in severe COVID-19 patients than in mild COVID-19 and normal controls.[29] Rodrigues *et al.,*2021 stated that both Casp1p20 and IL-18 correlated with the markers of COVID-19 severity, yet the severe cases of COVID-19 revealed higher levels of Casp1p20 but not IL-

18. On the contrary, levels of IL-18, but not Casp1p20, were higher in non-survivors of COVID-19 patients in comparison with the survivors.[12] On the other hand, Adamik *et al.,* 2022 showed that the levels of IL-18 in the groups of survivors and non-survivors did not differ significantly.[22]

In conclusion, our data indicated that inflammasome activation was a hallmark in COVID-19 patients, The markers of activation were positively correlated with many parameters of inflammation suggesting their important roles in the pathophysiology of the disease and its progression. IL-1β was the only marker to be correlated with disease severity and therefore may be suggested as a potential marker for identifying severe COVID-19 patients.

Author Contributions

SHA, RFA and NME contributed to the study conception and design. SKA, AMZ and OHB contributed to material preparation, data collection and analysis. DMM provided clinical support. SKA, OHB and NME wrote the manuscript draft. All authors read and approved the final manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

The study protocol was reviewed and approved for research ethics by the Institutional Review Board of the Faculty of Medicine, Assiut University (Dated November 2021).

Informed consent

All participants provided written informed consent before included in the study.

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