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Potential Role of Inflammasome NLRP3 and IL-1β Gene Expression in COVID-19 Patients: Impact of Ferritin and D –Dimer

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Abstract

Dysregulated inflammation and hypercoagulability underlie the development of severe illness in COVID-19. Biomarkers related to inflammasome activation, including interleukin (IL)-1β and NLRP3, and inflammatory lung cytokine as IL-8. As well as coagulation markers like D-dimer. The aim of this study was to identify associations between the dysregulated immune response biomarkers and the severity of the disease that will help in prognostic and potential therapeutic utility**.** Twenty five mild/moderate and 25 severe COVID-19 patients were included in this study as well as 25 healthy controls. Survival analysis was done after follow up for one month. Inflammasome (IL-1β, NLRP3), lung cytokine (IL-8), and vitamin D levels were analyzed by real time PCR and ELISA. The mRNA expression levels of IL-1β , NLRP3 and serum level of IL-8 were elevated in cases than controls. COVID-19 patients exhibited lower levels of vitamin D. The severe group showed significant higher levels of D dimer and ferritin. Correlation analyses indicated associations between IL-1β , NLRP3, IL-8, D-dimer and ferritin. ROC curve analyses demonstrated the potential of IL-1β , NLRP3 and IL-8 in distinguishing between mild/moderate and sever cases. Survival analyses indicated a link between high levels of IL-1β , NLRP3 and IL-8 with decreased survival.. The inflammasome appears pivotal in orchestrating detrimental hyperinflammation in severe COVID-19. IL-1β holds particular potential as a prognostic indicator of poor outcomes. In the future, the possible using of these biomarkers inhibitors may improve survival , decrease severity and improve management of the cases.

Keywords: COVOID-19, NLRP3, IL-8, IL-1β, vitamin D.

Full length article **Corresponding Author*, e-mail: tasneem.a.alsanory@gmail.com Doi # https://doi.org/10.62877/23-IJCBS-24-25-19-23

1. Introduction

The coronavirus disease 2019 (COVID-19) continues to cause substantial morbidity and mortality worldwide. While most infected patients develop mild symptoms, a subset progress to severe disease characterized by acute respiratory distress syndrome, systemic hyperinflammation, and multiple organ failure [1]. The dysregulated immune responses underlying the development of severe COVID-19 remain incompletely understood, though recent evidence indicates inflammasome activation may play a key role [2]. Inflammasomes are innate immune complexes that drive the maturation of pro-inflammatory cytokines interleukin (IL)-1β and IL-18 upon sensing pathogen- and danger-associated molecular patterns [3, 4]. The nucleotide-binding oligomerization domain (NOD)-like receptor family pyrin domain containing 3 (NLRP3) inflammasome, in particular, has been implicated in viral infections and the regulation of inflammatory lung disease severity. Activation of the NLRP3 inflammasome and production of IL-1β is regulated in part by the danger signal ferritin, an iron storage protein [5]. Simultaneously, dysregulated coagulation contributes to poor prognosis in COVID-19, with marked increases in D-dimer levels correlating with disease severity and mortality [6]. The purpose of this study is to examine the expression of NLRP3, IL-1β and serum level IL-8 and vitamin D ,and their relation with the levels of ferritin and D dimer in patients with COVID-19 at different severity level of the disease. The goal

is to understand the possible association between increased activity in inflammasome genes levels and ferritin and D dimer levels and the severity of COVID-19. Also, The possible inhibitors of these biomarkers may delay the progression of the disease. By conducting this research, gain insights into the mechanisms behind inflammation and identify markers that can help predict adverse outcomes in patients. Understanding these pathways that contribute to COVID-19 is crucial, for finding effective therapies and improving management of severe cases.

2. Materials and Methods

2.1 Study design

The current investigation is an observational cross-sectional study. Patients admitted to the Intensive Care Unit (ICU) at Assiut University Hospital provided the samples. The laboratory analysis was conducted at Assiut University's Medical Research Centre, Faculty of Medicine. Institutional review board (IRB) of Faculty of Medicine-Assiut University approval for this study was obtained under IRB local approval number 04-2023-200059. All participants or their relatives were provided a written informed consent form. The investigation was conducted in accordance with the Helsinki Declaration, clinical trial number (NCT06080750). Fifty patients were included in this study, 25 of them have mild to moderate COVID-19, 25 severe COVID-19 patients according to clinical and radiological criteria [7]. Follow up of the patients for 3 months or death was done. The inclusion criteria of being 18 or older, a positive RT-PCR test, lung involvement on CT imaging. Exclusion criteria included autoimmune diseases, immunosuppressive drug use, and other chronic inflammatory diseases. COVID-19 severity was categorized into mild, moderate, severe, and critical forms, based on symptoms and lung CT scans. SARS-CoV-2 detection was done using RT-PCR with nasopharyngeal swabs, RNA extraction through the RNA Jia Virus Kit, and qPCR with the COVITECH-COVID-19 Multiplex qPCR Kit. Twenty-five age and sex matched subjects were included as control group. Five ml bood samples was divided into 2 parts: The first part kept at -80 C for RNA extraction. While the second part allowed to clot for 2 hours at room temperature before centrifugation for 15 min at 1000×g at 2-8℃, Collect the serum to carry out the ELISA assay.

2.2 Evaluation of target gene expression NLRP3 and IL-1B

Quantification of relative gene expressions mRNA of NLRP3 and IL-1β was performed by using specific primers and Applied Biotechnology ABT 2X SYBR Green master mix. A 20 µL PCR reaction containing 10 µL SYBR Green qPCR Master Mix (2X), one µL of each primer forward and reverse of corresponding primer, template DNA (200 ng/reaction) and 2 µL of nuclease-free water was used to perform PCR. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the internal control and was incorporated for the normalization of mRNA expression. Using Real-Time PCR (7500 Fast Applied Biosystems, Germany) for Real-Time Quantitative polymerase chain reaction (RT-qPCR). The relative mRNA expression was calculated by the threshold cycle value according to the $2^{-\Delta\Delta CT}$ equation in each sample. The 5′ - 3 ′ primer sequences for the following primers were used; NLRP3 forward: GAGGAAAAGGAAGGCCGACA and reverse TGGCTGTTCACCAATCCATGA. IL-1β forward: GAGCAACAAGTGGTGTTCTCC and reverse AACACGCAGGACAGGTACAG. Housekeeping gene GADPH forward: CGTGGAAGGACTCATGACCA and reverse GGCAGGGATGATGTTCTGGA. The thermal cycler (Applied Biosystems 7500 fast Real-Time PCR Systems, Germany) was programmed to initial denaturation at 95°C for 5 min followed by 40 cycles at 95 °C for 45s, 60 °C for 45s (annealing temperature) and then 72 °C for 1min for NLRP3. Annealing temperature was 55 °C for 45s for IL-1B , 49˚C for 45 seconds for GAPDH.

2.3 Evaluation of serum vitamin D and IL-8 levels

Quantitative measurement of total vitamin D (25-hydroxycholecalciferol) was determined in serum by vitamin D ELISA kit (Cat. No. 30850) by the competitive immunoassay technique, supplied by Epitope Diagnostics Inc. (EDI™). Quantitative determination of Human IL-8 concentrations in serum was done using -Elabscience-IL-8. ELISA kit catalog number E-CL-H0048 depending on the Sandwich immune assay principle. ELISA plate provided in this kit has been pre-coated with an antibody specific to Human IL-8.

2.4 Statistical analysis

The analysis and management of data were performed using SPSS version 27.0. We divided the COVID-19 patients into two groups based on their severity status; mild/moderated and severe groups. To assess any differences between the variables we initially applied a normality test (Kolmogorov Smirnov) to the quantitative variables. For variables we presented them as median (interquartile range IQR) while utilizing the Mann-Whitney U test for comparison between three groups and Kruskal–Wallis test was used to compare between two groups. Categorical variables were expressed numerically as percentages. Compared statistically using both the chi square test and Fisher's exact test. ROC curve with odds ratio and 95% C.I. was used to show the cut off value of biomarkers to discriminate between mild/moderate and sever groups. Correlations between biomarkers was done by Pearson test. Regression analysis (univariate and bivariate) assesses the dependence of the disease severity on biomarkers. The survival analysis by Kaplan Meier test. Statistical significance was considered with a two P value less, than 0.05.

3. Results and Discussion

3.1. Demographic data in the three studied groups

The statistical analysis showed no difference in age between the control and cases (47.88 ±1.60; 50.23± 10.34). Also, no significant difference between mild and sever groups. The gender distribution, among the three groups did not show any differences (p = 0.387) as shown in Table 1.

3.2 Laboratory data in the two studied subgroups (mild and severe COVID-19 patients) Table 1

As regard blood gases, the severe cases showed significantly lower PaO2and oxygen saturation (p<0.001) than mild/ moderate cases . Additionally, the severe group had marked lymphopenia 0.67 x10^3/µL versus 1.08 x10^3/µL in mild/ moderate cases (P<0.001). Coagulation abnormalities were another feature distinguishing the severe phenotype, indicated by markedly higher INR. The deranged

electrolyte profile in severe cases, manifestinflammation marked by nearly double the C-reactive protein levels compared to the mild/moderate group ($p<0.001$). The findings revealed variations in the levels of D-dimer and ferritin between the two groups. The severe group exhibited higher levels compared to the moderate group ($p = 0.026$ and $p = 0.001$, respectively). Elevated D-dimer levels suggest a state of increased blood clotting. Are commonly associated with cases of COVID-19, as depicted in Figure 1A. On the other hand, heightened ferritin levels indicate inflammation and have been observed in severe COVID-19 cases, as shown in Figure 1B.

3.3 IL-1β , NRLP3, IL-8 AND vitamin D levels in the three studied groups of COVID-19 patients

As shown in Figures 1C, 2D and 2E there were variations, in the expression of IL-1B , NLRP3 and IL-8 among the control group /moderate COVID-19 group and severe COVID-19 group ($p<0.001$ for all comparisons). Specifically, although the mild/moderate group displayed higher expression levels of IL-1B and NLRP3 compared to the control group ($p>0.05$) ,the severe COVID-19 group exhibited the highest expression levels of these genes. These levels were significantly elevated compared to both the control group ($p<0.001$) and mild/moderate groups ($p<0.001$). Furthermore, Figure 1F demonstrates differences in vitamin D levels between the control group and COVID-19 groups. Vitamin D was notably reduced in both the mild/moderate ($p=0.002$) and severe ($p<0.001$) groups when compared to the control group; however no statistically significant difference was found in vitamin D levels, between the mild/moderate and severe disease groups ($p=0.883$). There is a correlation between IL-1β and ferritin and D-dimer ($r=0.325$,0.021; $r=0.383$; $p=0.006$;respectively). When considering NLRP3, there is a moderate positive correlation with ferritin ($r=0.481$, $p<0.001$), but no significant positive correlation with D-dimer ($r=0.182$, $p=0.206$). As for IL-8, it exhibits a moderate and positive relationship with ferritin ($r=0.577$, $p<0.001$). These findings are depicted in Table 2. The cut-off values of serum biomarkers can provide valuable insights into the severity of COVID-19 exhibited good discriminative capacity between severe and mild/moderate cases. Figure 2, demonstrated IL-1β , with an AUC of 0.846 ($p<0.001$). Its optimal cut-off points of ≥4.99 resulted in a sensitivity of 80.0% and specificity of 66.0%. NLRP3 also demonstrated an AUC of 0.787 ($p<0.001$). The optimal cut-off value of ≥**2.76** yielded a sensitivity of 76.0% and specificity of 68.0%. This indicates that elevated NLRP3 levels may be associated with an increased risk of severe COVID-19. IL-8 exceptional performance was reflected by an AUC of 0.96 ($p<0.001$), signifying its ability to perfectly distinguish between the two groups. The optimal cut-off point of ≥662.0 displayed impressive sensitivity and specificity, with values of 100.0% and 96.0% respectively. This suggests that IL-8 levels above this threshold may indicate a higher likelihood of severe disease. In contrast, Vitamin D, a crucial nutrient with immunomodulatory properties, demonstrated relatively lower discriminatory ability with an AUC of 0.633 ($p=0.084$).

3.4 Univariate and multivariate cox regression analysis of biomarkers associated with severity in covid-19 patients

Univariate Cox regression analysis showed that individuals with higher levels of biomarkers such as interleukin (IL) 1β, NLRP3 inflammasome, and IL-8 are more likely to experience severe COVID-19 symptoms Table 3. Among these biomarkers, IL-1β has the association with increasing the risk of disease by more than 16 times (HR 16.025, 95% CI 2.097, 42.471, $p = 0.008$). Similarly, elevated NLRP3 is associated with a 7-fold increased risk (HR 6.957 CI 1.566 30.908, $p = 0.011$), while higher levels of IL-8 are linked to an 8-fold rise in the likelihood of severe COVID-19 symptoms (HR 8.743 CI 1.956 39.066, $p = 0.005$). After adjusting for confounding factors in multivariate analysis, the significant association with prognosis remained for IL-1β (adjusted HR 8.401, $p = 0.045$), but not for NLRP3 and IL-8.

3.5 Survival analyses

The probability of survival, for COVID 19 patients whether their condition is severe or mild/moderate can be determined by analyzing the levels of IL-1β in their serum. In figure (3A) it was observed that patients with IL-1β levels below 4.99 had an estimated survival time of 26.870 days (with an error of 1.106 and a 95% confidence interval ranging from 24.703 to 29.037 days). On the hand patients with IL 1B levels to or higher than 4.99 had an estimated average survival time of 16.190 days (with a standard error of 2.231 and a 95% confidence interval ranging from 11.817 to 20.562 days). The p value for this group was found to be less than <0.001 indicating a correlation between levels of IL-1β and increased overall survival. Similarly, by examining the levels of NLRP3 in the serum the probability of survival in COVID 19, patients can also be assessed (as shown in Figure (3B). Patients with levels below 2.76 had an estimated overall survival time of approximately 25.957 days (with a standard error of around1.389 and a confidence interval ranging from approximately 23.234 to 28.679 days). Conversely patients with levels, to or higher than2.76 were observed to have an estimated mean overall survival time of about 14.570 days (with a standard error close to1.768 and a confidence interval ranging from roughly11.104 to18.036 days). The p value associated with this group was 0.003 suggesting a link, between levels of NLRP3 and improved overall survival. Figure (3C) demonstrates the likelihood of survival based on serum IL-8 levels in differentiating between mild/moderate COVID-19 patients. Patients with IL-8 levels below 662.0 had an estimated survival of 26.792 days with a standard error of 0.819 and a 95% confidence interval ranging from 25.187 to 28.396 days. On the hand patients with IL-8 levels to or higher than 662.0 had an average estimated overall survival of 13.583 days with a standard error of 1.885 and a confidence interval ranging from 9.889 to 17.277 days (at the significance level $p <0.001$). These findings indicate a significant correlation, between IL-8 levels and increased survival. We conducted a study to evaluate NLRP3 and IL-1β mRNA gene expression levels in COVID-19 patients, shedding light on the disease's molecular mechanisms. The study also examined the relationships between IL-8 and vitamin D levels and indicators of the inflammasome NLRP3 and IL-1β. The study examined these biomarkers' complex relationships with COVID-19 severity, patient survival, and clinical indications such ferritin and D-dimer. This study's complex network of correlations enhances the understanding of COVID-19's pathophysiological mechanisms and may lead to new therapeutic approaches. These biomarkers and

their inhibitors may help design better COVID-19 treatments by reducing disease severity and consequences. This holistic approach to analyzing COVID-19's molecular landscape highlights the disease's complexity and lays the groundwork for targeted therapies. Regarding the demographic and laboratory data of the patients were divided into mild/moderate and severe COVID-19 subgroups based on demographic and laboratory data. The severe group in COVID-19, marked by a higher mean age, showed significant differences in blood gases with lower pH, PaO2, and O2 saturation, indicative of severe respiratory compromise, consistent with findings in ARDS [8]. Blood analysis revealed lower lymphocyte counts and higher INR, aligning with COVID-19-induced lymphopenia and increased coagulation risk. This reflects the virus's impact on the immune system and coagulation pathways [9]. Electrolyte imbalances, notably lower sodium and higher potassium, were prominent in the severe group [10]. The elevated CRP indicated heightened inflammation, suggesting potential multiple organ dysfunction syndrome (MODS) involvement [11]. This study found a stepwise increase in IL-1β fold change moving from healthy controls (1.06 to mild/moderate COVID-19 patients (4.74, $p<0.001$) and finally to the severe disease group (13.29, $p<0.001$). This aligns with Paranga et al.[12], Bagheri-Hosseinabadi et al.[13], and Majeed et al.[1], where IL-1β similarly rose with advancing COVID-19 severity compared to controls. Adnan Mezher et al., [14]. and Lu et al. [15] also described persistently elevated IL-1β in COVID-19 patients over time.

The incremental IL-1β elevations likely reflect escalating inflammasome activation and hyperinflammation from cellular damage and viral sensing. SARS-CoV-2 may directly stimulate epithelial IL-1B release, which recruits more inflammatory cells in a positive feedback loop, as Monteagudo et al. [16] explained. The virus can also activate NLRP3 inflammasomes to cleave IL-1B production and disrupt IL-10 inhibition, enabling cytokine storm development [17, 18]. In the current study, we found significantly higher NLRP3 expression levels in COVID-19 patients compared to controls. The median NLRP3 level in controls was 0.90, compared to 2.53 in mild/moderate COVID-19 patients ($P<0.001$) and 5.53 in severe COVID-19 patients ($P<0.001$). Our results closely align with Houshmandfar et al., [3] showing a similar marked ~6-fold upregulation of NLRP3 in COVID-19 patients versus controls ($p<0.001$), confirming significant NLRP3 induction. In contrast, Bagheri-Hosseinabadi et al. [13] found a non-significant difference, which may stem from viral evasion of NLRP3 signaling early on, as Zheng et al. [19] described, coupled with NLRP3 levels rising later with worsening inflammation regarding van den Berg and Te Velde [2]. SARS-CoV-2 can directly activate NLRP3 through ACE2 binding, ROS release, and ion fluctuations. Virus-induced interferons, cytokines, cellular stress from hypoxia/metabolic disturbances, and genetic NLRP3 variants may also modulate expression in COVID-19 [3, 5, 20, 21].

In our results, IL-8 levels showed a clear association with COVID-19 severity increase from controls (389.99 pg/mL) to mild/moderate (623.73 pg/mL) and severe (883.56 pg/mL) COVID-19 groups ($p<0.001$). This aligns with Bergantini et al., Ma et al., Xu et al, Liu et al., and Kwon et al [22-26], where IL-8 similarly rose with escalating disease severity, often affectedly in critically ill patients. As a potent neutrophil chemoattractant, higher IL-8 likely underlies pathological neutrophilia and infiltration in severe COVID-19. Barnes et al. [27] proposed IL-8 helps recruit polymorphonuclear myeloid-derived suppressor cells, which suppress virus-targeting T cells to perpetuate SARS-CoV-2 infection [28]. According to the results of this study significantly lower vitamin D levels in COVID-19 groups, especially severe cases, versus healthy controls ($p<0.001$). This aligns with the meta-analysis by D'Ecclesiis et al [29]. indicating vitamin D supplementation may reduce COVID-19 severity and mortality. However, other studies comparable Dean, [30] and Lin et al.,[31] did not find significant correlations between vitamin D status and COVID-19 susceptibility or outcomes. While deficiency was more common among COVID-19 positive individuals in the latter study, the difference was not statistically significant. The correlation analysis revealed significant positive associations between inflammatory (IL-1β , NLRP3, IL-8), iron storage (ferritin), and coagulation (D-dimer) markers in COVID-19 patients. We found correlations between ferritin and IL-1β /D-dimer levels, suggesting heightened inflammation and clotting activation with rising ferritin. D-dimer additionally correlated with IL-1B , while NLRP3 associated with IL-1B and IL-8. These relationships align with Vargas-Vargas and Cortés-Rojo [32], who also reported direct ferritin correlations with IL-1B and D-dimer during COVID-19. However, Onuk et al. [4] and Bagheri-Hosseinabadi et al. [13] implicate found NLRP1 and ASC have a more significant role generating active IL-1B compared to NLRP3. The analysis found inflammatory cytokines IL-8, IL-1B , and NLRP3 demonstrated the best performance differentiating severe versus mild/moderate COVID-19. IL-8 showed superior discriminative ability with AUC 0.960 and cutoff ≥662 pg/mL, detecting severe cases with 100% sensitivity and 96% specificity. Aligning with Shafiek et al., IL-1β also exhibited good diagnostic accuracy (AUC 0.846). Furthermore, the extensive cytokine panel in Majeed et al. [1] identified some markers comparable to our IL-8, while we found better sensitivity/specificity. Compared to Bergantini et al., [22] The IL-8 cut-off provided higher AUC (0.960 vs 0.88) and sensitivity (100% vs 66%), suggesting improved precision identifying severe presentations. The survival analysis revealed striking prognostic ability of IL-1β , NLRP3, and IL-8 levels to differentiate mild/moderate versus severe COVID-19 outcomes. Patients with higher biomarker levels showed significantly shorter mean survival times ($p<0.001$ all). Aligning with these findings, Onuk et al.[4] highlighted IL-1β 's association with mortality in regression models. However, Majeed et al. [1] did not find significant relationships for some markers, likely reflecting statistical methodology differences. Importantly, Valle et al. [6] verified inflammatory cytokines' independent predictive strength for survival, even after adjusting for confounders.

Table 1: Demographic and laboratory data in the two studied subgroups: mild and severe COVID-19 patients

Variables		Mild/moderate group (n = 25)	Severe group (n = 25)	P value
Age Mean ± SE. Median (IQR)		47.64 ±2.52 46.0 (40.0-55.0)	52.54 ±8.01 54.50 (44.0-60.0)	**N.S**
Gender Number (%)	female	17 (68%)	13(52%)	**N.S**
	Male	8(32%)	12(48%)	
Blood gases Mean ± SE. Median (IQR)	PH	7.40 ±0.01 7.40 (7.40-7.45)	7.50 ±0.01 7.50 (7.50-7.52)	**<0.001***
	PaCo2	42.40 ±3.89 38.00 (33.00-52.00)	45.65 ±2.60 50.00 (45.00-58.00)	N. S
	PaO2	72.84 ±1.20 72.00 (70.00-80.00)	58.50 ±0.78 59.50 (55.00-60.00)	**<0.001***
	O2sat	87.00 ±0.89 85.00 (85.00-90.00)	71.73 ±1.90 70.00 (70.00-80.00)	**<0.001***
	HCO3	23.50 ±1.83 22.00 (20.00-28.40)	22.10 ±0.67 23.00 (22.00-24.00)	N. S
Blood picture Mean ± SE. Median (IQR)	HCT	41.00 ±2.61 44.00 35.00 49.00	40.85 ±2.51 43.00 35.00 49.00	N. S
	lymphocytes	1.08 ±0.07 1.00 (0.90-1.30)	0.67 ±0.07 0.58 (0.44-0.87)	**<0.001***
	INR	1.10 ±0.02 1.08 (1.05-1.10)	1.76 ±0.12 1.68 (1.18-2.07)	**<0.001***
Minerals Mean ± SE. Median (IQR)	Na	139.92 ±1.87 139.00 (135.00-143.00)	127.12 ±1.44 127.00 (120.00-130.00)	**<0.001***
	K	3.46 ±0.17 3.30 (3.20-4.00)	4.97 ±0.08 4.70 (4.70-5.50)	**<0.001***
	Ca	8.56 ±0.12 8.50 (8.50-9.40)	8.07 ±0.22 7.30 (7.00-9.40)	N. S
	Mg	2.48 ±0.19 1.80 (1.60-2.30)	2.49 ±0.14 2.10 (1.80-3.30)	N. S
CRP Mean ± SE. Median (IQR)		64.44 ±6.88 55.00 (44.00-80.00)	116.07 ±10.37 107.85 (100.00-139.00)	**<0.001***

 # Kruskal–Wallis test was used to compare difference in means between groups.

p: p value for comparing between the two studied groups; *Statistically significant at $p \leq 0.05$;

IQR: **Inter quartile range ;** SE: **Standard error**,

N.B : Liver and kidney functions within normal range in all groups

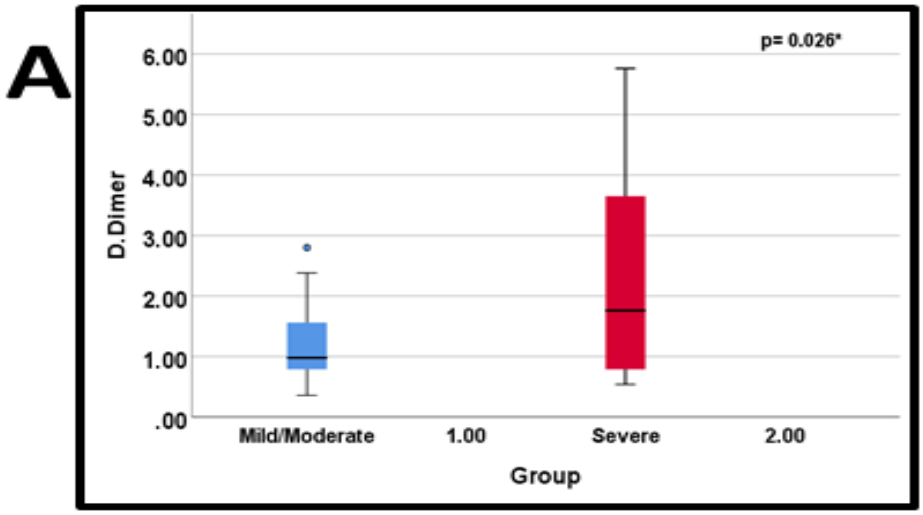
N.S: Non-significant

Table 2: Correlation of D -dimer and ferritin with studied biomarkers

Variables		Ferritin	D-dimer	IL-1B	NLRP3
IL-1B	r value	0.325	0.383		
	p value	0.021*	0.006*		
NLRP3	r value	0.481	0.182	0.777	
	p value	<0.001*	0.206	<0.001*	
IL-8	r value	0.577	0.266	0.832	0.768
	p value	<0.001*	0.062	<0.001*	<0.001*

Table 3: Univariate and multivariate COX regression analysis of biomarkers associated with severity inCOVID-19 patients

	Univariable HR	**95% C.I**		**P value**	**Multivariable HR**	**95% C.I**		**P value**
		lower	**upper**			**lower**	**upper**	
	16.025	2.097	42.471	**0.008***	8.401	1.053	67.029	**0.045***
NLRP3	6.957	1.566	30.908	**0.011***	2.764	0.595	12.849	0.195
IL-8	8.743	1.956	39.066	**0.005***	4.208	0.896	**IL-1B**	0.069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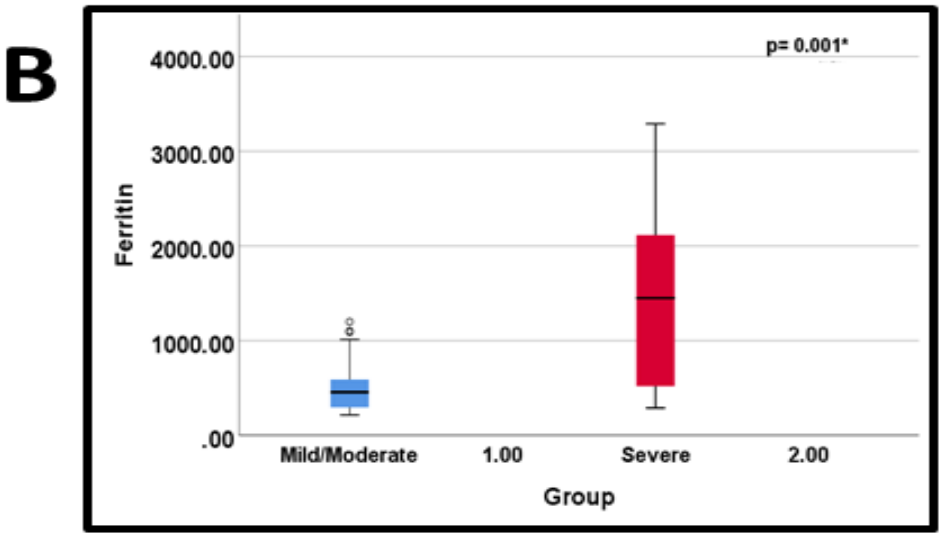
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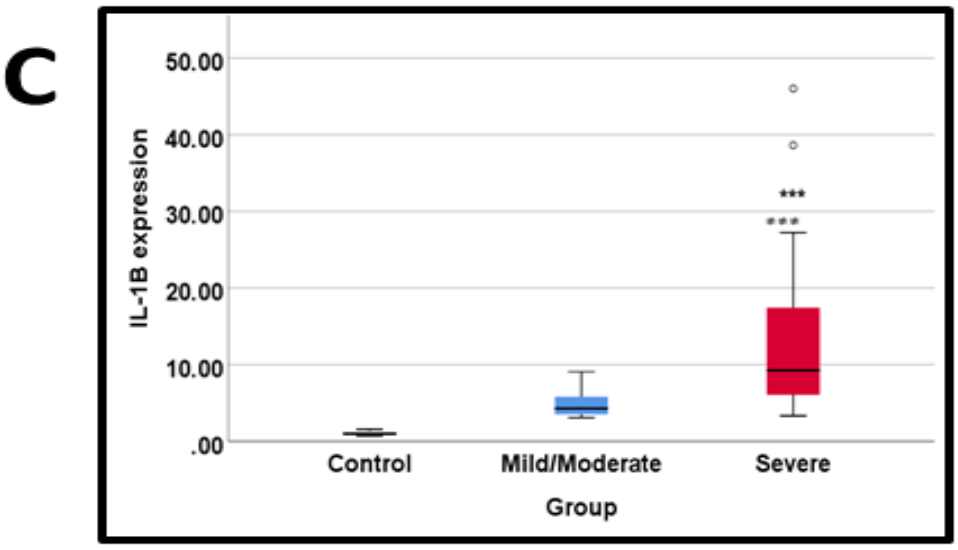
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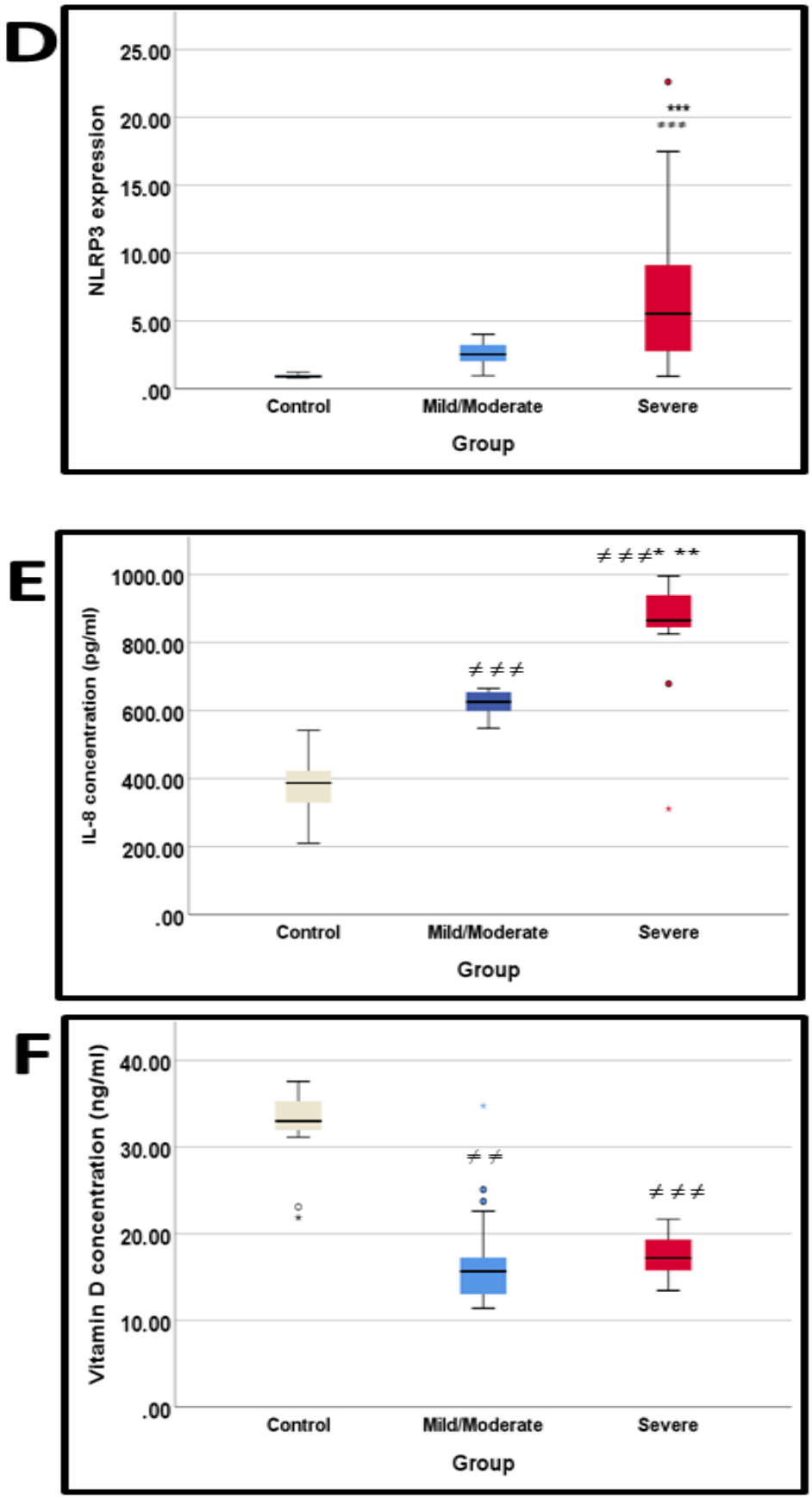
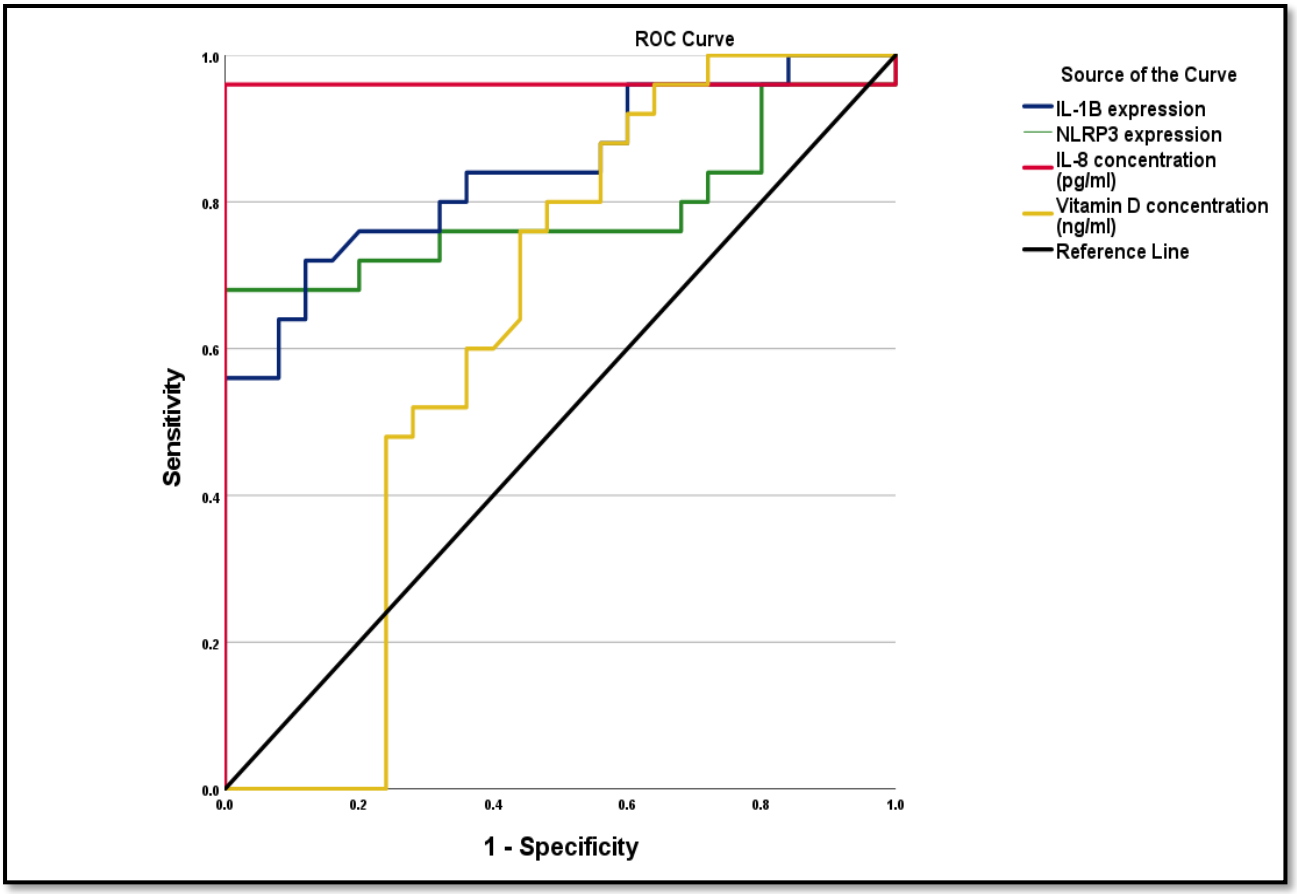


Figure 1: Levels of D-dimer, ferritin and biomarkers in studied groups. A) D-dimer, B) ferritin, C) IL1B relative expression, D) NRLP3 relative expression, E) serum level of IL8 and F) Vitamin D.



	AUC	P value	95% CI		Cut off point	Sensitivity	Specificity
			lower	upper			
IL-1B	0.846	**<0.001**	0.737	0.955	**≥4.99**	80.0%	66.0%
NLRP3	0.787	**<0.001**	0.647	0.927	**≥2.76**	76.0%	68.0%
IL-8	0.960	**<0.001**	0.883	0.989	**≥662.0**	100.0%	96.0%
Vitamin D	0.633	0.084	0.468	0.798	16.25	60%	54%

Figure 2: Receiver operating curve (ROC) curve of the serum biomarkers to discriminate severe from mild/moderate COVID-19 patients.

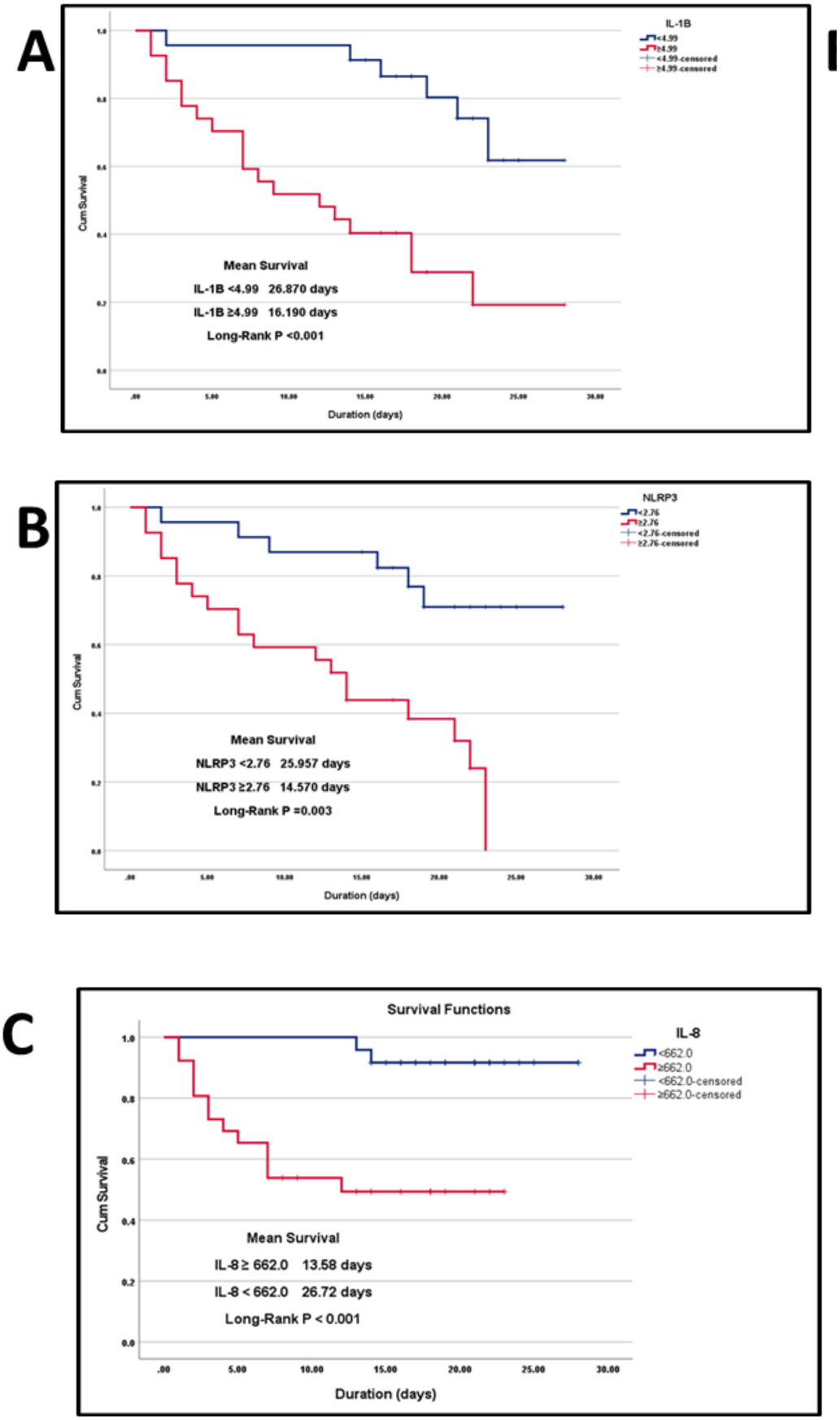


Figure 3: Survival rate analysis by Kaplan-Meir curve analysis, A) IL-1B of COVID-19 patients, B) NLRP3 of COVID-19 patients, C) IL-8 of COVID-19 patients.

4. Conclusions

In conclusion, this research suggests that IL-1β , NLRP3 and IL-8 could serve as biomarkers linked to the severity of COVID 19. Higher levels of these markers are associated with outcomes like impaired oxygenation, abnormal blood clotting and widespread inflammation. Additionally, lower levels of these markers are linked to improved survival rates. Both univariate and multivariate Cox regression analyses support the value of IL-1β , NLRP3 and IL-8 in determining the severity of COVID 19. These findings highlight the significance of these biomarkers, in evaluating disease severity guiding treatment choices and predicting patient outcomes.

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Data availability

The data will be available on request.

Conflict of interest

The authors declare no conflicts.

Ethical approval

The Institutional review board (IRB) of Faculty of Medicine-Assiut University approval for this study was obtained under IRB local approval number 04-2023-200059.

Informed consent

A written informed consent forms were provided by all participants or their relatives.

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