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Prognostic value of IL18 serum levels in COVID-19 Patients at Beni- Suef University Hospital

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Abstract:

A cross-sectional, analytical study conducted over a period of 6 months from April to September 2021 after the approval of REC on 50 patients with confirmed positive of COVID-19 by SARS-CoV-2 nucleic acid RT-PCR recruited from Isolation departments and intensive care units (ICUs), Beni-Suef University hospital. The goal of this study was to assess the serum level of (IL-18) as a biomarker of COVID-19 disease progression. All participants underwent complete blood count (CBC), C- reactive protein (CRP), fibrinogen, D-dimer and liver enzymes and measurement of serum IL- 18 level. All patients had been followed until hospital discharge or death. Forty-two patients (84%) recovered and discharged from the hospital while eight cases (16%) died due to different etiologies: 2 patients due to suppurative lung infection, another 2 because of multi organ failure, 2 patients owing to respiratory failure and alveolar damage, and the last 2 patients with cardiac comorbidity after ischemic heart disease and hypertension.

There were a significant higher level of CRP, D-dimer, ferritin, and IL-18 among died as compared with recovered COVID-19 patients. Platelets count was significantly higher among recovered as compared with died COVID-19 patients. Correlation test showed a significant positive strong linear relationship between CRP level and IL-18, D-dimer level, and IL-18, and between ferritin level and IL-18. High values of IL-18 in patients' serum helped to assess the poor prognosis which was statistically significant with 81.5% sensitivity and 57.5% specificity at a cutoff point level ≥ 230.75. Elevated serum levels of IL-18 were associated with fatal

outcome in the COVID-19 infected patients and showed strong correlation with other inflammatory markers.

Keywords: COVID-19, IL-18, Biomarkers.

1. Introduction

Coronaviruses (CoVs) are responsible for life- threatening out-breaks; including severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS) and lastly Coronavirus Disease 2019 (COVID-19) [1]. COVID-19 is a rapidly spreading global threat declared as a pandemic by the WHO [2]. COVID-19 is transmitted via droplets or direct contact. It infects the respiratory tract resulting in pneumonia in most cases and a serious lung inflammation, acute respiratory distress syndrome (ARDS) [3], myocardial and renal injury [4] and thrombotic manifestations especially in elderly patients and those with chronic comorbid conditions such as diabetes mellitus, hypertension and heart failure [5].

COVID-19 is caused by the SARS-CoV-2 that belongs to the beta-coronaviruses subfamily. Coronaviruses are enveloped, positive single - stranded large RNA viruses [6]. Although the first data available about COVID-19 indicates possible animal-to-human transmission via wild animals in Huanan seafood Market in Wuhan [7], epidemiological data and studies after that have increasingly demonstrated that the virus transmits human-to-human [8]. and confirmed to be spread through respiratory droplets from coughs or sneezes [9], [10] with the ability of the host to shed the infection while asymptomatic [11]. Studies are now also proposing the possible feco-oral transmission of the virus [12_14].

According to the Coronavirus Disease 2019 (COVID-19) Treatment Guidelines, patients with COVID-19 disease are roughly divided into five groups:

Asymptomatic, **Mild Illness, Moderate Illness**, **Severe Illness and Critical Illness** [15].

Several alterations in cytokine network occur during COVID-19 infection including but not limited to; increased plasma levels of Interleukins (IL)-1β, IL-6, IL-7, IL-8, IL-10, IL-18, granulocyte colony-stimulating factor (G- CSF), and tumor necrosis factor-alpha (TNF-α) [16]. Moreover, it was found that plasma concentrations of these cytokines in ICU patients are higher than in non-ICU patients [17]. Therefore, it has been suggested that exuberant cytokine production “cytokine storm” is the main cause of tissue injury leading to ARDS, multi-organ failure and death in COVID-19 [18].

The catastrophic clinical condition in COVID-19 shares similar features with macrophage activation syndrome (MAS) encountered in several clinical conditions, such as adult-onset Still's disease (AOSD), systemic lupus erythematosus, Epstein-Barr virus (EBV) and influenza which should be immediately recognized and treated for its rapidly fatal course [19]. The Pathogenesis of MAS is complicated and has not been solved yet. IL-1, IL-6, IL-8, IL-10, IL-18, interferon (IFN)-γ and TNF-α are the important cytokines responsible for MAS development [20]. Amongst these, the most studied one is IL-6 which is increased both in mild and severe COVID-19 patients and correlated with the pulmonary infiltration area in patients with ARDS [21]. IL-18 is produced by macrophages at the very early stages of viral infections and induces production of IL-6 and IFN-γ. Which are considered critical for optimal viral host defense [22]. However, aberrant IL-18 production can also lead to severe pathological injury. The activity of IL-18 is balanced by IL-binding protein (IL-18BP) which is stimulated by IFN-γ, prompting a classical feedback loop whereby IL-18BP offsets exuberant IL-18 and attenuates the IFN-γ response [23]. Markedly elevated serum IL-18 levels have been linked to disease severity and mortality in some viral infections characterized by cytokine storm such as avian influenza and Dengue virus [24]. Upon viral infection, IL-18 release induces ferritin, explaining the frequently observed hyper-ferritinemia in viral infections [25]. Identification of the role of IL- 18 will shed light on the disease pathogenesis of COVID-19 which is also characterized by hyper-ferritinemia and cytokine storm. Moreover, serum concentrations of IL-18 might serve as a biomarker to predict COVID-19 disease outcome. The current study was designed to assess serum level of IL-18 as a biomarker of COVID-19 disease progression.

2. Patients and Methods:

Patients:

The present study is a cross-sectional analytical one, conducted over a period of 6 months from April to September 2021 after the approval of REC. It had been held at Chest department and Microbiology and Immunology Department at faculty of medicine Beni-Suef University. The study population enrolled were all probable cases of COVID-19 attending Beni- Suef University Hospital during the study period a total sample size of 50 patients with positive clinical and radiological findings of COVID-19 were recruited from isolation departments and ICUs at Beni-Suef University Hospital and were estimated for 90% power, α- error probability 0.05 and 10% dropout rate during follow up and a 30 healthy individuals age- and sex-matched were used as a control group.

Methods:

Laboratory investigation of the study group (cases) had been retrieved from their files; [complete blood count (CBC), blood biochemistry C-reactive protein (CRP), fibrinogen, D-dimer, ferritin, and liver enzymes (AST and ALT)]. From all patients, 2 samples were collected: nasopharyngeal swab for the detection of COVID-19 viral RNA by RT-PCR, and a venous blood sample for determination of serum level of IL-18. While control group was assessed for CRP, D-dimer, ferritin, and serum level of IL-18.

Ethical Consideration:

All the participants were informed about the procedures regarding the study. They had been notified of their rights to refuse participation or withdraw from the study without having to give reasons. Participants were guaranteed anonymity and all information provided would be treated with confidentiality.

The required administrative regulations were fulfilled. The ethical approval of the faculty of medicine, Beni-Suef University research ethical committee (REC) was obtained prior to the beginning of the work.

Statistical Analysis of data:

Statistical analysis was done using statistical package for social sciences (SPSS) computer software (version 25), IBM software, USA. Categorical variables were described as the total number and percentage for each category, whereas continuous variables were described as the mean ± standard deviation and Median (IQR) values were specified for continuous variables that did not conform to the normal distribution. Normally distributed continuous variables were compared with the student's t- test, non-normally distributed continuous variables were compared with the Mann– Whitney U test and categorical variables were compared with the chi-square test. Pearson's correlation analysis; was done to evaluate linear relationship between IL-18 with other studied parameters in all patients. Correlation was considered significant at $P < 0.05$. Correlation is considered positive (direct correlation) when r (correlation coefficient) had a + signal and negative (inverse correlation) in case of – signal and it is considered: (weak when $r = >0 – 0.35$, moderate when $r = >0.35 – 0.65$; and strong when $r = > 0.65$). Uni-variable analysis was performed in all variables to determine statistically significant factors that may have contributed to in-hospital mortality of COVID-19 patients. The discriminatory ability for IL-18 to predict mortality in COVID-19 patients was determined using the receiver operating characteristic (ROC) curve. These results were reported as area under the curve (AUC) and 95% confidence intervals (CI). A cut-off value was defined as that with the highest validity. For quantitative parametric data:

Independent samples t test was used to compare quantitative measures between two independent (cases and control) groups.

3. Results:

The current study is a cross-sectional analytical study included 50 patients (24) males and (26) females with age ranged from 23 to65 and an average age of (45.26 ±10.95) years old. From the studied fifty patients, (13, 26%) were smokers, while (37, 74%) were non- smokers. Regarding the associated co-morbidities, twenty patients (40%) were hypertensive, and twenty-three patients (46%) were diabetic.

Table (1): Inflammatory markers and Laboratory assessment in the studied population, (N= 50):

	Minimum	**Maximum**	**Mean**	**Std. Deviation**
CRP, mg/L	3.11	210.00	99.74	66.49
D-Dimer, ng/Ml	0.18	11.30	4.25	4.35
Ferritin, µg/L	17.00	1012.00	359.19	388.59
Hemoglobin, g/dL	8.00	17.00	12.35	1.88
WBC /mm3	2.80	15.80	7.43	3.14
Neutrophils /mm3	1.30	14.50	5.83	3.04
Lymphocytes /mm3	0.30	6.30	1.18	0.96
Platelets /mm3	118.00	475.00	245.66	92.84
LDH, U/L	245.00	1372.00	513.18	221.29
AST, U/L	14.00	142.00	48.04	26.57
ALT, U/L	6.00	196.00	38.84	36.30
IL-18, pg/mL	34.40	380.00	124.38	95.60

(CRP): C-reactive protein, (WBC): white blood cell, (LDH): Lactate dehydrogenase, (ALT): Alanine aminotransaminase, (AST): aspartate aminotransferase, (IL-18): interleukin-18.

Figure (1) demonstrates the distribution of the studied population by their outcome. Forty-two patients (84%) recovered and discharged from the hospital while eight cases (16%) died due to different etiologies: 2 patients due to suppurative lung infection, another 2 because of multi organ failure, 2 patients owing to respiratory failure and alveolar damage, and the last 2 patients with cardiac comorbidity after ischemic heart disease and hypertension.

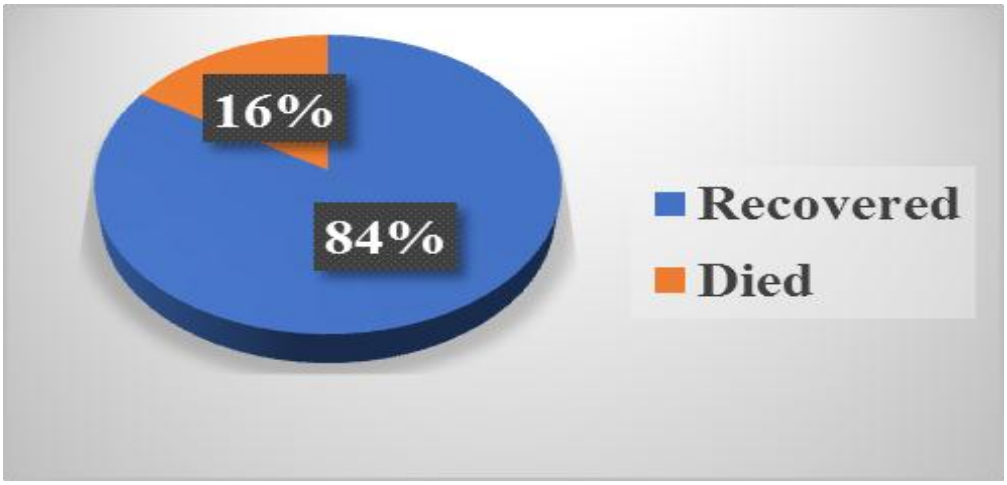

Table (2) demonstrates the association between sociodemographic data, associated co-morbidities, inflammatory biomarkers, and laboratory investigations with COVID-19 disease outcome:

		Outcome		**P-value**
		Improved N= 42	**Died N= 8**	
Gender	Male, N= 24	19 (45.2)	5 (62.5)	0.305
	Female, N= 26	23 (54.8)	3 (37.5)	
Age	Mean ±SD	44.80 ±10.95	47.62 ±11.33	0.434
HTN	Normotensive, N= 30	29 (69.0)	1 (12.5)	0.005*
	Hypertensive, N= 20	13 (31.0)	7 (87.5)	
DM	Nondiabetic, N= 27	26 (61.9)	1 (12.5)	0.013*
	Diabetic, N= 23	16 (38.1)	7 (87.5)	
Smoking	Non-Smoker, N= 37	33 (78.6)	4 (50.0)	0.109
	Smoker, N=13	9 (21.4)	4 (50.0)	
CRP, mg/L	Mean ±SD	84.93 ±60.14	177.50 ±39.23	0.001*
D-Dimer, ng/mL	Mean ±SD	3.02 ±3.58	10.71 ±0.53	0.001*
Ferritin, µg/L	Mean ±SD	262.56 ±341.31	866.50 ±156.73	<0.001*
Hemoglobin, g/dL	Mean ±SD	12.28 ±1.98	12.72 ±1.28	0.414
WBC /mm3	Mean ±SD	7.33 ±2.93	7.93 ±4.28	0.748
Neutrophils /mm3	Mean ±SD	5.71 ±2.76	6.45 ±4.41	0.771
Lymphocytes /mm3	Mean ±SD	1.20 ±1.04	1.03 ±0.38	0.417
Platelets /mm3	Mean ±SD	315.37 ±120.95	232.38 ±81.70	0.012*

LDH, U/L	Mean ±SD	495.30 ±189.68	607.00 ±346.53	0.181
AST, U/L	Mean ±SD	47.64 ±26.51	50.12 ±28.64	0.283
ALT, U/L	Mean ±SD	41.02 ±39.11	27.37 ±9.37	0.379
IL-18, pg/mL	Mean ±SD	92.33 ±62.42	292.68 ±51.22	<0.001*

Table (3): Correlation between the inflammatory markers, laboratory assessment tests and IL-18 serum Level Correlation test showed a significant positive strong linear relationship between CRP level and IL-18, D- dimer level and Ferritin level.

		IL_18
Age	Pearson Correlation	0.156
	p-value	0.278
CRP, mg/L	Pearson Correlation	0.757**
	p-value	0.000
D-Dimer, ng/mL	Pearson Correlation	0.814**
	p-value	0.000
Ferritin, µg/L	Pearson Correlation	0.822**
	p-value	0.000
Hemoglobin, g/dL	Pearson Correlation	0.190
	p-value	0.186
WBC /mm3	Pearson Correlation	-0.040
	p-value	0.782
Neutrophils /mm3	Pearson Correlation	0.007
	p-value	0.962
Lymphocytes /mm3	Pearson Correlation	-0.049
	p-value	0.735
Platelets /mm3	Pearson Correlation	-0.320
	p-value	0.063
LDH, U/L	Pearson Correlation	0.270
	p-value	0.058
AST, U/L	Pearson Correlation	0.097
	p-value	0.501
ALT, U/L	Pearson Correlation	-0.194
	p-value	0.178

Figure (2): The results of ROC curve analysis of IL-18 in the expired vs. recovered COVID-19 patients.

Receiver operating characteristic (ROC) curve analysis was used to assess the discriminative ability of IL-18 for outcome among died as compared with recovered COVID-19 patients. The results of IL-18 (ROC) curve analysis showed p-value <0.05 so; the serum ratio assessed the poor prognosis with a statistically significant level with 81.5% Sensitivity (true positive cases) and 57.5% Specificity (true negative cases) at a cutoff point level ≥ 230.75.

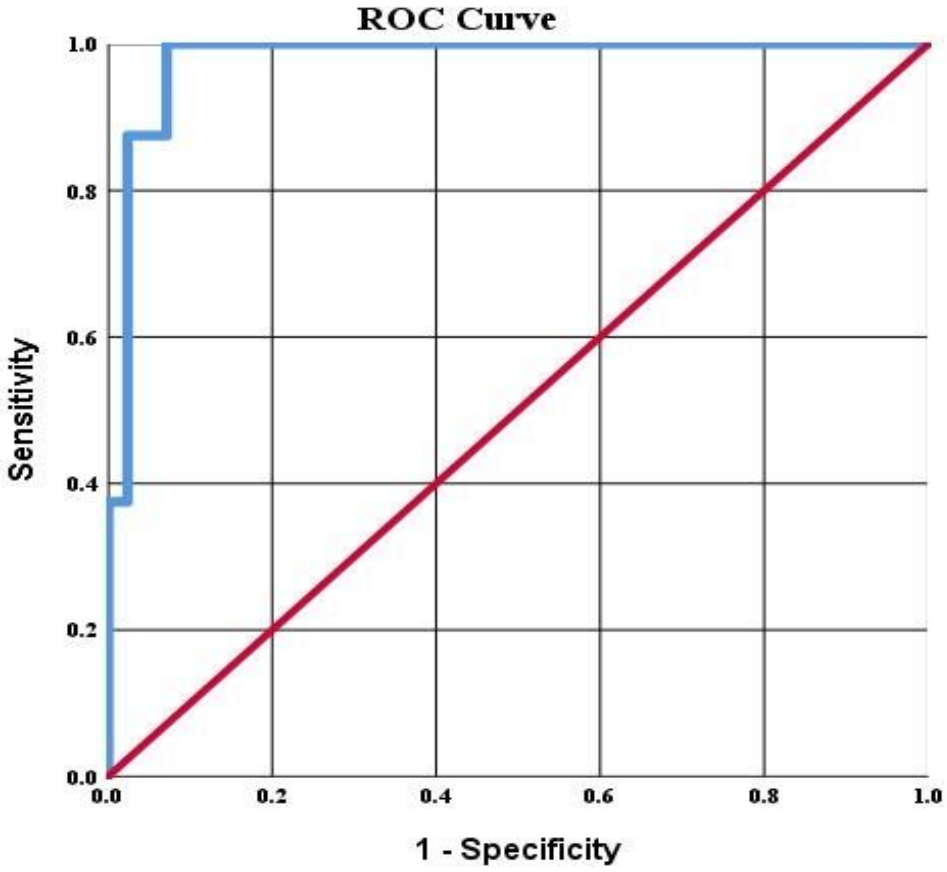

Table (4): Comparisons between inflammatory, laboratory assessment tests and IL-18 serum in different study groups (cases & control). The table illustrates that there was highly statistical significance difference between cases and controls as regards (CRP-ferritin-D-dimer and serum IL-18) with p-value <0.05.

Parameters	Cases (n=50)	Control (n=30)	P-value	Sig.
CRP	99.75 ± 66.49	2.70 ± 1.44	<0.001	HS

D-dimer	4.25 ± 4.35	0.26 ± 0.11	<0.001	HS
Ferritin	359.20 ± 388.59	83.27 ± 67.30	<0.001	HS
IL18 in serum	124.39 ± 95.61	65.97 ± 25.46	<0.001	HS

4. Discussion:

Biomarkers that can provide a guide to disease progression, prognosis, and seriousness are of great significance [26]. IL-18 is produced by macrophages at very early stages of viral infections and induces production of IL-6 and IFN-γ which are considered critical for optimal viral host defense [27]. Health practitioners are looking for an easily available and low-cost prognostic marker to determine those who would progress to serious conditions among the patients of COVID-19. Therefore, over a period of six months, the current cross-sectional analytical study was conducted on 50 patients (24 males and 26 females) with confirmed positive COVID-19 by SARS-CoV-2 nucleic acid RT-PCR recruited from Isolation departments and intensive care units (ICUs), with Microbiology & Immunology Department at faculty of medicine Beni-Suef University to assess serum level of (IL-18) as a biomarker of COVID-19 disease prognosis. Another 30 healthy individual's age and sex matched group with comparison between the cases regarding different inflammatory (CRP-ferrtin-d-DIMER-IL-18).

COVID-19 can occur in any age group. Our data showed that the participants' age ranged from 23 to 65 with an average age of (45.26 ±10.95) years old. Died patients were relatively older as compared with recovered patients, however this difference was statistically non-significant (p=0.43). A meta-analysis conducted by Yang et al., suggest that age and comorbidities are highly related in COVID-19 patients [28].

In our study there were 24 male patients &26 female patients. It was found in previous meta-analysis conducted with an objective to compare the epidemiological variations in COVID-19 patients reported in studies from inside and outside of China that the male population has a higher proportion in all included studies, suggesting a higher prevalence of the disease in the male population. Another previous meta- analysis has found similar results while studying the gender in COVID-19 [28] & [29], however in the present study, males and females were nearly equally presented without a statistically significant difference in their outcome, it is possible to interpret this difference as small

sample size in the current study, as well as being a one-center study.

Table (2) in our study demonstrates the association between socio demographic data, associated co-morbidities, inflammatory biomarkers and laboratory investigations with COVID-19 disease outcome. Died males were more than females (5 vs. 3) but, without a statistically significant difference, (p=0.305).

Died patients were relatively older as compared with recovered patients (47.62 ±11.33 vs. 44.80±10.95), however this difference was statistically non-significant (p=0.434). Hypertensive patients showed worse outcome, of the eight cases who died, seven had hypertensive disorders with a statistically significant (p-value= 0.005). Diabetic patients showed worse outcome, of the eight cases who died, seven had diabetes mellitus with a statistically significant (p-value= 0.013). Smoking showed non-statistically significant difference in relation to the outcome. CRP was significantly higher among died as compared with recovered COVID-19 patients (177.50±39.23 vs. 84.93 ±60.14, p=0.001).

D-Dimer was significantly higher among died as compared with recovered COVID-19 patients (10.71 ±0.53 vs. 3.02 ±3.58, p=0.001). Ferritin was significantly higher among died as compared with recovered COVID-19 patients (866.50 ±156.73 vs. 262.56 ±341.31, p=0.001).

Platelets was significantly higher among recovered as compared with died COVID-19 patients (315.37 ±120.95 vs. 232.38 ±81.70, p=0.012). IL-18 was significantly higher among died as compared with recovered COVID-19 patients (292.68 ±51.22 vs. 92.33 ±62.42, p=0.001).

Other inflammatory markers showed non-statistically significant difference between recovered and died COVID-19 patients.

Early and current scientific reports have identified the presence of one or more coexisting comorbidities, particularly in severe COVID-19 patient groups [30], [31]. In the current analysis, our results confirmed what was published before in COVID-19 context, that many of infected people who were hospitalized suffered from at least one comorbidity (40% were hypertensive, and 46% were diabetic), and that death rates were significantly related to the presence of these comorbidities, out of the expired cases, 7 (87.5%) had HTN disorders with a statistically significant (0.005) difference and, 7(87.5%) had DM with a statistically significant difference, and those results are in complete agreement with [30-27]. Patients with underlying HTN, CAD, HF, DM, obesity, COPD, and CKD experienced a higher risk of mortality compared to patients without these comorbidities [32]. Mechanisms by which these comorbidities may affect the outcome of COVID-19 disease are unclear. Nonetheless,

comorbidities could modify risks of infection, severity of COVID-19 clinical course, and response to administered therapies [33].

Smoking is among the risk factor for progression of COVID-19 [34], however, there was a high degree of heterogeneity amongst studies evaluating current smoking, even when analyzing good-quality studies only. For patients with a smoking history, there is an increased risk of presentation to hospital with severe, as well as severe or critical, COVID-19 and subsequent increased risk of in-hospital mortality [35]. In the current study the prevalence of smoking among studied participants was (26%). Reported smoking prevalence in the current study was higher than the reported in many other studies, (ranged from 3.7% to 16.8%), [36]. This difference could be related to the possibility for poor recording or under-reporting of the smoking status, lack of adjustment for confounding factors and potential differences in healthcare access between smokers and non-smokers [37].

Another argument that has been suggested is that hospitalized COVID-19 cases are more likely to suffer from smoking-related comorbidities and might have already quit smoking because of these comorbidities. While this is a possibility, population surveys show that comorbidities, such as COPD, are still more prevalent in current rather than former smokers [38].

In the current study, the routine laboratory findings provide important insights on the strong association between the elevated level of CRP, D-dimer, Ferritin, and IL-18 with the mortality rate of COVID-19. Also, platelets play an essential role in blood coagulation, immunity, angiogenesis, and inflammation. Thrombocytopenia occurs due to many factors, e.g., Hematopoietic cells were directly inhibited by coronavirus. as well as damage to pulmonary tissue and endothelial cells can cause platelet activation, accumulation, and retention in the lungs, as well as thrombus formation at the damaged area, leading to increased platelet consumption [39]. In the current analysis, platelets were significantly lower among died COVID-19 patients ($p=0.012$). The lower platelet count has been reported to be a marker of poor prognosis, not only in COVID-19 patients but also in different population of critically ill patients [40]. In line with our findings, Zhao et al., Lippi et al., (2020) with poor prognosis in covid-19 patients documented that thrombocytopenia was associated with enhanced risk of severe COVID-19 and mortality [8] & [41], [42]. Probable causes of platelet changes in COVID-19 patients might be a direct invasion of hematopoietic cells or bone marrow stromal cells by corona virus or the lung injury prevents release of platelets from mature megakaryocytes as lung might be one of the organs where it occurs [5] & [8].

Markedly elevated serum IL-18 levels have been linked to severe disease and mortality in some viral infections characterized by cytokine storm such as avian influenza and Dengue virus [43], [44]. In this study, we found that serum IL-18 concentration is remarkably increased in patients with COVID-19 and observed that IL-18 was significantly higher among expired as compared with recovered COVID-19 patients (292.68 ±51.22 vs. 92.33 ±62.42, p=0.001). Our finding came in accordance with that of **Kerget and his colleagues** (2021), who reported elevated IL-18 levels among non-survivors compared to surviving patients in their study that was conducted to investigate the relationships between clinical course, prognosis, and mortality in 100 COVID- 19 patients. They concluded that IL-18, IL-1Ra, and alpha defensin are important players in the inflammatory/anti-inflammatory balance in viral infections [45].

In a study conducted on 58 COVID-19 patients to investigate the association of IL-18 with the other inflammatory markers and disease severity and prediction of prognosis, they found that IL-18 level on admission was continuously increasing across the severity groups, and it was highest in those who had worse outcome [21].

Consistent with previous SARS-CoV-2 studies [21] & [45] we observed that IL-18 was strongly correlated with ferritin. Upon viral infection, IL-18 release induces ferritin, explaining this strong correlation and the frequently observed hyperferritinemia in viral infections [25]. Also, IL-18 was strongly correlated with CRP and D- dimer which are highly predictive of worse prognosis. IL-18 might serve as a biomarker to predict disease outcome, the optimal cutoff value of IL- 18 for mortality prediction in the present study was ≥ 230.75 pg/mL with 81.5% Sensitivity and 57.5% Specificity. This cut-off was lower than the 576 pg/mL reported by Satış et al., with a sensitivity of 78% and specificity of 77%, however, both studies concluded that IL-18 is an important risk factor related to severe COVID-19 and/or in-hospital mortality. Comparisons between inflammatory, laboratory assessment tests and IL-18 serum in different study groups (cases &control). There was highly statistical significance difference between cases and controls as regards (CRP-Ferritin-d-Dimer and serum IL-18) with p-value <0.05. We have several limitations within the study, first the number of patients; particularly severe ones were relatively small to draw clear conclusions, second, number of patients who had worse outcome was relatively small, and finally the serum IL-18 concentrations were measured only at admission and serial measurements with certain time periods might better clarify its role on disease outcome.

5.Conclusion:

Serum IL-18 concentrations have a significant correlation with the COVID-19 severity and other inflammatory markers, we report three major findings from the current study; first patients with elevated serum IL-18 levels had higher incidences of fatal outcomes. Second, serum IL-18 concentration was positively correlated with other known biomarkers Better characterization of the role of IL-18 could constitute a therapeutic avenue to the treatment of COVID-19.

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