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Interleukin-35, D-dimer, and ferritin as mortality predictive in SARS-CoV-2

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ABSTRACT

Background: Knowing which patients would be more likely to die is crucial for making better use of the little resources available. To do this, the ability of several inflammatory indicators to identify mortality outcomes has been detected. **Materials and methods:** A total of 125 severe/critical COVID-19 patients were involved in this work divided into two groups based on survival, dead group (62 patients) and live group (63 patients). Between March 2022 and July 2022, these patients were admitted to Marjan medical city and Al-Sadeq hospital. Patients were determined as severe cases according to the guidelines released by National Health World. The inflammatory cytokine (IL-35) was detected by the ELISA technique. **Results:** IL-35 showed no statistical differences between lived 6.85 (5.44- 8.72) ng/ml and dead 6.53 (5.82- 7.89) ng/ml patients (p= 0.79). D dimer, and ferritin increased significantly in dead patients 2467.5 (1368. 7- 3697) ng/ml and 1621.5 (792.3- 2359) ng/ml respectively compared to live patients 557 (430- 689) ng/ml and 268 (186- 449) ng/ml respectively (P < 0.0001). The ROC or area under the curve (AUC) for D-dimer was 0.89 with high sensitivity (95%) and specificity (77%). Ferritin also showed a large AUC that was 0.90 with high sensitivity and specificity (95%) and (81%) respectively. The cut off point for both D- dimer and ferritin was 693.67 ng/ml and 475 ng/ml respectively. **Conclusions:** IL-35 revealed no significant differences between dead and lived patients with COVID-19 (p = 79). Positive strong correlation observed between ferritin and D-dimer (r= 0.85, p< 0.0001). No correlation was found between IL-35 and both ferritin and D-dimer (p > 0.05).

Introduction

The severe acute respiratory syndrome-coronavirus flare-ups in both 2002 and 2003, as well as the Middle East Respiratory Syndrome-coronavirus (MERS) outbreak in 2012, showed the scope for newly developing coronaviruses to be transmitted from animal to the human and individual to another [3]. HCoV229E, HCoV-OC43, MERS-CoV, SARS-CoV, HKU1, and SARS-CoV-2 are among the seven human coronaviruses (HCoVs) presently known [1-3].

Coronavirus disease 2019 (COVID-19), due to contagion with severe acute respiratory

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syndrome coronavirus 2 (SARS-CoV-2) that stated as a pandemic lately, majority of cases are self-limiting; nonetheless, it can proceed to a severe form with a high death rate at any time [4, 5].

IL-35 reduces inflammatory responses by modulating different cytokines and thereby controlling STAT signaling, unlike IL-12 and IL-23 [6]. As a result, IL-35 can activate STAT1 and STAT4 in T cells and trigger STAT1 and STAT3 in B cells when it binds to IL-35R [7]. IL-35 suppresses the development of monocyte-derived dendritic cells (MoDCs) by activating the STAT 1/3 pathways while concurrently suppressing the p38 MAPK and NF-κb signaling pathways, reducing pro-inflammatory activities [8].

Interleukin-35 (IL-35) is a newly discovered heterodimeric cytokine that belongs to the IL-12 family. It works as an inhibitory cytokine in the immune system, modulating malfunctioning T cells, and regulating various immune-related inflammatory factors. [9,10]

Interleukin-35 is also thought to have a function in the regulation of autoimmune illnesses, inflammatory diseases, bacterial and viral infectious diseases, and malignancies. Recent IL-35 research, along with new methodologies for examining receptors and signal transduction pathways, enables IL-35 to be considered as a potential immunotherapy target [11,12]. The aim of the current study was to detect the relation of IL-35 to the outcome of COVID-19 as well as other inflammatory biomarkers such as D-dimer and ferritin.

Materials and methods

A total of 125 severe COVID-19 patients were recruited in this study; they were all hospitalized to the COVID-19 wards of Al-Sadeq hospital and Marjan Medical City from 3/3/2022 to 24/7/2022. They were between the ages of 16 and 90. Each patient in this investigation had reverse transcriptase-polymerase chain reaction results that were positive for SARS-CoV-2, which supported the diagnosis of COVID-19 (RT-PCR). Patients divided to two groups based on survive; 62 dead group and 63 live group. Based on the SpO2 percentage, patients were classified as severe or critical (90%) [13].

Each patient whose IL-35 level was measured had blood and serum samples taken. BT LAB Company ELISA kits and a Biotek EL800 automated immunoassay analyzer were used to measure IL-35 (BioTek, USA). Biochemical tests assessed by using the automated Architect Ci 8200 system (Abbott Diagnostics, Lake Forest, IL, USA). Biochemical parameters were detected by reagents, calibrators, and controls depending on given manufacturer's instructions.

The Babylon Health Directorate approved the moral position. Before taking the sample, permission from the patient and his relative was sought. In addition to sampling, safety and health precautions were implemented.

Data were analyzed by SPSS for windows version 26 for statistical analysis (GraphPad Software, San Diego, California, USA). The outcomes were presented as median (25th–75th interquartile range, IQR). The Mann–Whitney U test was used to compare two groups. P value < 0.05 was taken into account to denote statistical significance. Additionally, Spearman correlation test used to explain the connection between pro-inflammatory cytokine serum levels IL-35, D-dimer and ferritin. The ROC or area under the curve (AUC) used to detect sensitivity and specificity.

Results

IL-35 showed no statistical differences between lived patients 6.85 (5.44- 8.72) ng/ml and dead 6.53 (5.82- 7.89) ng/ml (p = 0.79). D dimer, and ferritin increased significantly in dead patients 2467.5 (1368. 7- 3697) ng/ml and 1621.5 (792.3-2359) ng/ml respectively compared to live patients 557 (430- 689) ng/ml and 268 (186- 449) ng/ml respectively (p < 0.001) **(Table 1).**

The ROC or area under the curve (AUC) for D-dimer was 0.89 with high sensitivity (95%) and specificity (77%). Ferritin also showed large AUC that was 0.90 with high sensitivity and specificity (95%) and (81%) respectively. The cutoff value for both D- dimer and ferritin was 693.67 ng/ml and 475 ng/ml respectively **(Table 2) (Figure 1 and 2).**

Positive strong correlation observed between ferritin and D-dimer (r= 0.85, p < 0.0001). No correlation was found between IL-35 and both ferritin and D-dimer (p> 0.05) **(Table 3).**

Table 1. Distribution of variables according to status of patients (Live or Dead).

Variables	Status of patients	Number	Median (IQR) Or NO (%)	*P*. value
IL-35	Live	63	6.85 (5.44- 8.72)	0.79
	Dead	62	6.53 (5.82- 7.89)	
D- dimer	Live	63	557 (430- 689)	< 0.001
	Dead	62	2467.5 (1368. 7- 3697)	
Ferritin	Live	63	268 (186- 449)	< 0.001
	Dead	62	1621.5 (792.3- 2359)	

Table 2. Sensitivity, specificity, and cut point for different inflammatory variables.

Variables	AUC	Std. Error	Sig	Lower bond	Upper bond	Cut point	Sens.	Spec.
D- dimer	0.898	0.028	<0.001	0.843	0.953	693.67	0.952	0.778
Ferritin	0.902	0.028	<0.001	0.847	0.957	475.00	0.952	0.810
IL-35	0.486	0.053	0.79	0.382	0.590	10.2350	0.143	1.000

Figure 1. Area under the curve for D- dimer, and ferritin.

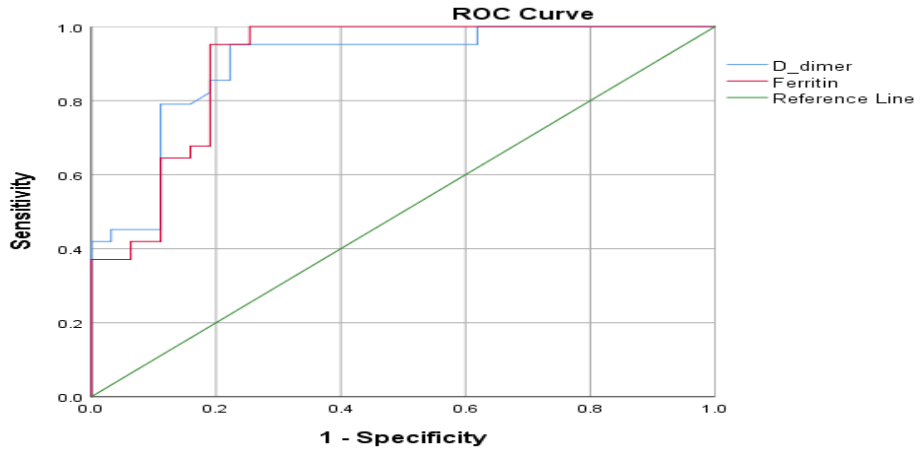
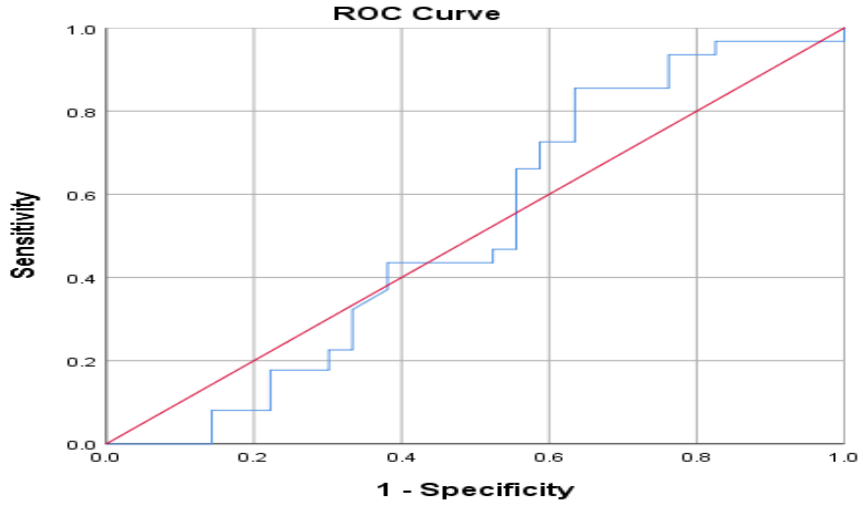


Figure 2. Area under the curve for presepsin, and IL-35.



Discussion

Sometimes, the immune system's reaction to a viral infection might be more destructive than the virus itself [14,15]. In order to manage COVID-19, immunopathogenesis must also be taken into account in addition to adaptive immunity. Since there have only been a few trials on IL-35, it is currently unclear how it works to fight viral infection [16].

To more effectively use the limited resources available, it is essential to be able to identify which patients would have a higher chance of dying. In order to do this, a number of inflammatory markers have been investigated to determine how well they can forecast mortality outcomes [17].

The current study found that IL-35 has no statistical differences between lived patients 6.85 (5.44- 8.72) ng/ml and dead 6.53 (5.82- 7.89) ng/ml (p= 0.79). D dimer, and ferritin increased significantly in dead patients 2467.5 (1368. 7- 3697) ng/ml and 1621.5 (792.3- 2359) ng/ml respectively compared to live patients 557 (430- 689) ng/ml and 268 (186- 449) ng/ml respectively ($p < 0.001$).

In a comprehensive study, individuals with COVID-19 who had acute respiratory distress syndrome (ARDS) and the subset of those who had died had significantly higher blood D-dimer levels [18]. The scientists came to the conclusion that there was a direct relationship between high D-Dimer levels and COVID-19-related morbidity and death. Greater blood D-dimer levels were substantially linked to COVID-19 mortality. Hence, the monitoring D-Dimer in COVID-19 patients may have some significant therapeutic effects to help customize the treatment as well as the greater than abnormal blood D-dimer levels were seen in 63.9% of the living patients and 96% of the patients who passed away, respectively ($p = 0.002$) [19].

According to a meta-analysis, individuals with COVID-19 were more likely to die, develop ARDS, and require ICU care when their ferritin and D-dimer levels were increased in their serum [20]. Other explanations for the increases in these inflammatory indicators include bacterial infections and subsequent viral infections. Thus, it may be advised to use these indicators to track the development and improvement of COVID-19 patients [19].

In our study, the ROC or area under the curve (AUC) for D-dimer was 0.89 with high sensitivity (95%) and specificity (77%). Ferritin also showed large AUC that was 0.90 with high sensitivity and specificity (95%) and (81%) respectively. The cutoff value for both D- dimer and ferritin was 693.67 ng/ml and 475 ng/ml respectively.

Another study found that optimal cutoffs of ferritin (714.3 ng/mL) and D-dimer (2.1 mg/L) revealed AUCs ≥ 0.99 for in-hospital mortality. Patients with a ferritin ≥714.3 ng/mL had 3.7 (95% CI: 2.8–4.8) higher odds of in-hospital mortality compared to those with a lower ferritin value [21].

Another study mentioned the cutoff values for the parameters were 33.55 mg/L for CRP, 0.635 μ/L for D-dimer, and 263.5 U/L for LDH, each with high sensitivity and specificity [22].

Serum ferritin is an iron storage protein with the primary role of regulating cellular oxygen metabolism. It is composed of two different subunits, H and L. Previous studies have suggested H-ferritin acts as an immune modulatory molecule with both proinflammatory and immunosuppressive functions. Increased ferritin levels could be indicative of a strong inflammatory reaction related to viral entry into the human body and its impact on iron metabolism [23].

D-dimer is a degradation product of cross-linked fibrin, indicating increased thrombin generation and fibrin dissolution by plasmin. High D-dimer levels are common in acutely ill individuals with a number of infectious and inflammatory diseases [24]. Studies have indicated that the coagulation system is active in critically ill patients, and D-dimer levels correlate with activation of the pro-inflammatory cytokine cascade [25]. It is believed that coagulation abnormalities related to elevated levels of D-dimer cause venous thromboembolism, which may contribute to respiratory deterioration related to COVID-19 infection [26].

Conclusion

IL-35 revealed no significant differences between dead and lived patients with COVID-19 (p = 79). Positive strong correlation observed between ferritin and D-dimer (r= 0.85, $p< 0.0001$). No correlation was found between IL-35 and both ferritin and D-dimer ($p > 0.05$). The cutoff value for

both D- dimer and ferritin was 693.67 ng/ml and 475 ng/ml respectively.

Conflicts of interest

Not declared.

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