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Role of Biochemical and Hematological Markers in Predicting COVID-19 Severity and Mortality among Egyptian Patients.

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ABSTRACT:

Objectives: Coronavirus Disease 2019 (COVID-19) has a significant impact on global health, leading to severe disease and high mortality rates. Understanding COVID-19 severity and its associated mortality is crucial for managing the pandemic and developing effective treatments. Patients with COVID-19 show a wide array of clinical symptoms. Laboratory investigations, including several biochemical and hematological tests, are routinely needed to guide diagnosis and management. This study aims to evaluate the utility of specific markers, including procalcitonin (PCT), ferritin, D-dimer, interleukin-6 (IL-6), C-reactive protein (CRP), total lactate dehydrogenase (LDH) activity, lymphocyte count, and neutrophil-to-lymphocyte ratio (NLR), in predicting the severity and mortality of COVID-19 infection.

Patients and methods: This study was undertaken at Alexandria university main hospital representing 180 COVID-19 RT-PCR positive patients after approval of the Ethical Committee of the Medical Research Institute and exclusion of patients with chronic lung diseases, diabetes mellitus, renal failure, hypertension, malignancy and previous history of chemotherapy and radiotherapy. The patients were grouped according to National Institutes of Health (NIH) guidelines; as mild to moderate (n=80) and severe to critical (n= 100). All routine laboratory analysis was carried out as part of baseline assessment. Biomarkers of inflammation and infection were tested via the measurement of CRP, IL-6, PCT and ferritin. In addition to urea , creartinine, AST, ALT, LDH and complete blood count as well as INR and D-Dimer. Serum IL-6 and PCT concentrations were estimated by electrochemiluminscence assay, while total LDH activity was measured using kinetic colorimetric assay. D-Dimer was measured by particle‑enhanced immunoturbidimetric assay. Serum CRP and ferritin concentration was estimated using an immunoturbidimetric assay.

Results: Biomarker levels varied significantly among the subgroups, with the highest concentrations observed in patients admitted to the intensive care unit (ICU). Based on the AUC-ROC analysis, Serum IL-6 demonstrated the highest predictive power for identifying the need for ICU treatment, followed by D-dimer and CRP while mortality was most accurately predicted by serum LDH followed by D-Dimer, neutrophils/ lymphocytes ratio, IL-6 and ferritin.

Conclusion: Our study emphasizes the value of routinely available biochemical and hematological tests in managing severe COVID-19. Elevated baseline levels of these biomarkers suggest a higher likelihood of severe infection and an increased risk of mortality.

Keywords: COVID-19, SARS-CoV-2, interleukin-6, D-dimer, procalcitonin, ferritin

INTRODUCTION

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), first identified in Wuhan, China, in December 2019, rapidly became a global concern. This enveloped, single-stranded RNA virus, belonging to the family *Coronaviridae* and the subfamily *Orthocoronavirinae*, was identified as the causative agent of the pandemic. The SARS-CoV-2 infection has a dramatic impact on health with variable outcomes. It affects not only the lung but also other organs, such as gastrointestinal tract, kidney, liver, central nervous system and cardiovascular system. Factors influencing severity include age, underlying health conditions, and socio-economic status [(1).]

Individuals with comorbid conditions, including cardiovascular disease, obesity and diabetes show an increased risk of experiencing severe outcomes. The interplay of these factors creates a need for stratified care approaches based on patient risk profiles.[(2)]

Laboratory markers play a pivotal role in assessing COVID-19 severity and predicting outcomes. Commonly studied markers include C-reactive protein (CRP), lymphocyte count, neutrophil / lymphocyte ratio, D-dimer, and inflammatory cytokines. Elevated CRP levels often indicate systemic inflammation and are associated with more severe disease[(1)] . Lymphopenia is frequently observed in severe cases and reflects a compromised immune response [(3)] .

Cytokine profiles, particularly elevated levels of interleukins (IL-6, IL-10) in addition to tumor necrosis factor-alpha (TNF-α), indicate a hyper-inflammatory

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response known as a cytokine storm, which is associated with severe disease progression and respiratory failure. This hyper-inflammatory state necessitates careful monitoring and may guide therapeutic interventions. Acute respiratory distress syndrome (ARDS) is associated with the excessive production of inflammatory cytokines, leading to multiorgan dysfunction in viral infections, including COVID-19[(4)].

Procalcitonin (PCT) is a commonly used inflammatory marker in routine clinical practice. Any microbial infection can lead to a significant rise in PCT levels, as endotoxins and pro-inflammatory cytokines stimulate its release from parenchymal tissues. Numerous studies have supported that a marked increase in PCT levels from baseline indicates the onset of the critical COVID-19 status [(5)].

D-dimer has emerged as a significant predictor of mortality. Elevated levels are linked to thrombosis and increased risk of pulmonary embolism, contributing to the higher mortality rates seen in severe cases. Other coagulation markers, such as fibrinogen and prothrombin time, have also been studied, suggesting that coagulopathy is a critical aspect of severe COVID-19 [(6).]

The use of biomarkers for risk stratification allows for the picking out patients indicated for early intervention and intensive monitoring

2. Subjects and Methods

2.1. Study design and subjects:

This study was undertaken at Alexandria university main hospital representing 180 COVID-19 RT-PCR positive patients after approval of the Ethical Committee of the Medical Research Institute and exclusion of patients with chronic lung diseases, diabetes mellitus, renal failure, hypertension, malignancy and previous history of chemotherapy and radiotherapy. The patients were grouped according to NIH guidelines; [(7)] as mild to moderate (n=80) and severe to critical(n=100).

Patients in the mild group (Group I) were managed with home quarantine. Those in the moderate group (Group I) were admitted to the hospital and treated in isolation wards. Severe to critical cases (Group II), needed intensive care unit (ICU) admission. The patients were further categorized into survivors and non-survivors regarding mortality at the time of discharge for subsequent analysis.

2.2. Data collection

Clinical data collected included gender, age, admission time, and discharge time. Routine biochemical and hematological tests were carried out to assess the baseline status of the patients.

2.3 Methods:

Biomarkers of inflammation and infection including CRP, IL-6, PCT and ferritin. In addition to urea , creartinine, AST, ALT, LDH and complete blood count as well as INR and D-Dimer were assessed. Serum CRP and ferritin concentration was estimated using an immunoturbidimetric assay, while serum urea and creatinine levels and LDH, AST and ALT activities was analyzed by kinetic colorimetric assay. Serum IL-6 and PCT were assessed using electrochemiluminscence. Complete blood count (CBC) was performed on the Sysmex XN-550 cell counter. The haemoglobin concentration, haematocrit value, red and white cell counts as well as platelet count were measured, along with automatic calculation of the hematological indices. A blood smear was spread on a glass slide, left to dry, and stained with Leishmann stain for the determination of differential white cell count.x D-dimer level was measured by particle‑enhanced immunoturbidimetric assay. PT was measured and INR was calculated using CN-3000.

2.4. Statistical analysis

Data were analyzed using IBM SPSS v.16.0 statistical software. The normality of the distribution was assessed using the Kolmogorov-Smirnov test. Continuous variables with non-parametric distribution were expressed as the median (25th percentile, 75th percentile), while mean values with standard deviation were used for continuous variables with a normal distribution. Categorical variables were summarized as frequencies and percentages. The Student's unpaired t-test and Mann-Whitney U test were employed for two-group comparisons of continuous variables based on severity and mortality. Statistical significance was considered when $p < 0.05$. Receiver Operating Characteristic (ROC) curve analysis was performed to evaluate the diagnostic utility of various biomarkers in determining ICU admission and predicting mortality. Measures of diagnostic accuracy, including sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated using MedCalc's diagnostic test evaluation calculator.

3. Results:

3.1. Patients charcteristics

A total of 180 patients were included, 42% (n=76) were males and 58% (n=104) were females. The patients were catogorized according to disease severity as group I (n=80) and group II (n=100). The mean age of group I was 40.28 ± 15.51years while in group II was 53.36 ± 13.24 years. 28% (n=50) died of the disease among our study population. Table (1) shows the distribution of the studied patients according to COVID-19 illness severity. Table (2) shows comparison between mild/moderate and severe/critical COVID-19 patients according to demographic data .There was a statistically significant difference was observed in the age distribution between the two groups, with older individuals exhibiting higher disease severity.

Table (1): Distribution of the studied patients according to COVID-19 illness severity (n = 180).

Severity	n	%
Mild/ Moderate COVID-19 patients	**80**	**44.4**
Mild	46	25.6
Moderate	34	18.9
Severe/ Critical COVID-19 patients	**100**	**55.6**
Severe	56	31.1
Critical	44	24.4

Table (2): Comparison between mild/moderate and severe/critical COVID-19 patients according to demographic data.

	Mild/Moderate COVID-19 patients (n = 80)		Severe/ Critical COVID-19 patients (n = 100)		Test of Sig.	p
	No.	%	No.	%		
Gender						
Male	38	47.5	38	38.0	χ^2= 0.822	0.365
Female	42	52.5	62	62.0		
Age (years)						
Min. – Max.	19.0 – 75.0		29.0 – 80.0		t= 4.317*	<0.001*
Mean ± SD.	40.28 ± 15.51		53.36 ± 13.24			

3.2. Biochemical and hematological biomarkers

Table (3) shows comparison between mild/moderate and severe/critical COVID-19 patients according to the measured biomarkers. All biomarkers were distributed in a statistically significant (p<0.001) manner amongst the groups.

Table (3): Comparison between mild/moderate and severe/critical COVID-19 patients according to different biomarkers

Parameter	Mild/Moderate COVID-19 patients(n = 80)	Severe/ Critical COVID-19 patients(n = 100)	U	p
Aspartate aminotransferase (U/L)				
IQR	24.0 – 33.0	37.0 – 69.0	359.00*	<0.001*
Median (Min. – Max.)	31.0 (17.0 – 52.0)	47.0 (15.0 – 306.0)		
Alanine aminotransferase (U/L)	26.0 – 36.5			
IQR	26.0 – 36.5	34.0 – 90.0	473.50*	<0.001*
Median (Min. – Max.)	30.0 (12.0 – 49.0)	48.0 (15.0 – 459.0)		
Lactate dehydrogenase (U/L)				
IQR	316.5 – 410.0	390.0 – 510.0	493.0*	<0.001*
Median (Min. – Max.)	370.0 (265.0 – 610.0)	470.0 (290.0 – 1214.0)		
Ferritin (ng/ml)				
IQR	61.0 – 343.5	460.0 – 758.0	179.0*	<0.001*
Median (Min. – Max.)	160.0 (8.0 – 836.0)	610.0 (215.0 – 2127.0)		
Procalcitonin (ng/ml)				
IQR	0.04 – 0.10	0.20 – 0.41	239.50*	<0.001*
Median (Min. – Max.)	0.07 (0.01 – 0.30)	0.30 (0.05 – 1.60)		
Interleukin-6 (pg/ml)				
IQR	1.9 – 4.3	26.0 – 90.0	20.0*	<0.001*
Median (Min. – Max.)	2.75 (1.10 – 39.0)	54.0 (6.70 – 310.0)		
C-reactive protein (mg/L)				
IQR	2.8 – 18.7	48.0 – 109	69.0*	<0.001*
Median (Min. – Max.)	8.05 (0.70 – 118.0)	54.0 (18.0 – 151.0)		
D-Dimer (ng/ml)				
IQR	134.5 – 222.5	800.0 – 2000.0	45.0*	<0.001*
Median (Min. – Max.)	168.0 (88.0 – 879.0)	1500.0 (196.0 – 2300.0)		
International normalized ratio (INR)				
IQR	1.01 – 1.03	1.0 – 1.1	390.50*	<0.001*
Median (Min. – Max.)	1.02 (1.01 – 1.90)	1.05 (1.01 – 1.70)		

Table (4):Comparison between mild/moderate and severe/critical COVID-19 patients according to complete blood count (CBC) parameters.

CBC parameters	Mild/Moderate COVID-19 patients (n = 80)	Severe/ Critical COVID-19 patients (n = 100)	Test of Sig.	p
Hemoglobin (g/dl)				
Min. – Max.	10.80 – 16.80	8.80 – 16.20	t=	0.005*
Mean ± SD.	13.59 ± 1.56	12.59 ± 1.67	2.880*	
Platelets (×10³/µl)				
Min. – Max.	131.0 – 439.0	20.0 – 427.0	t=	0.047*
Mean ± SD.	248.5 ± 68.19	212.3 ± 95.78	2.014*	
***White blood cells* (×10³/µl)**				
IQR	4.36 – 7.40	8.10 – 15.3	U=	<0.001*
Median (Min. – Max.)	6.25 (3.39 – 27.20)	11.10 (3.50 – 25.40)	348.5*	
Neutrophils (×10³/µl)				
IQR	1.9 – 4.5	6.1 – 13.5	U=	<0.001*
Median (Min. – Max.)	3.41 (0.97 – 24.40)	9.46 (2.40 – 24.70)	198.0*	
Lymphocytes (×10³/µl)				
IQR	1.5 – 2.5	0.52 – 1.6	U=	<0.001*
Median (Min. – Max.)	1.94 (0.49 – 5.72)	0.91 (0.20 – 2.70)	352.00*	
Monocytes (×10³/µl)				
IQR	0.35 – 0.60	0.21 – 0.42	U=	<0.001*
Median (Min. – Max.)	0.47 (0.23 – 2.07)	0.30 (0.05 – 1.42)	512.50*	
Basophils (×10³/µl)				
IQR	0.03 – 0.06	0.02 – 0.06	U=	0.121
Median (Min. – Max.)	0.04 (0.02 – 0.10)	0.04 (0.0 – 0.09)	789.0	
Eosinophils (×10³/µl)				
IQR	0.05 – 0.17	0.03 – 0.15	U=	0.112
Median (Min. – Max.)	0.09 (0.02 – 0.35)	0.06 (0.0 – 0.40)	804.50	
Lymphocytic count	**No. (%)**	**No. (%)**		
Normal ≥1.5 (×10³/µl)	27 (67.5%)	13 (26.0%)	χ^2=	<0.001*
Lymphopenia <1.5(×10³/µl)	13 (32.5%)	37 (74.0%)	15.500*	
Neutrophils/ lymphocytes ratio				
IQR	0.88 – 2.83	6.04 – 16.88	U=	<0.001*
Median (Min. – Max.)	1.50 (0.26 – 14.97)	8.85 (2.50 – 123.5)	121.0*	

The AUC-ROC curves were used to compare the diagnostic potential of different biomarkers including PCT, D-Dimer, IL-6, CRP, Ferritin and LDH to predict COVID-19 severity, respectively (Figures 1 and 2). Accordingly, serum IL-6 demonstrated the highest predictive power for ICU admission followed by D-Dimer, CRP and neutrophils/ lymphocytes ratio.

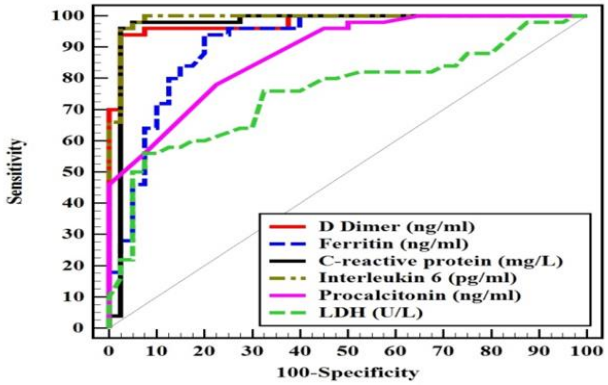

Figure (1): ROC curve for different markers to predict ICU admission.

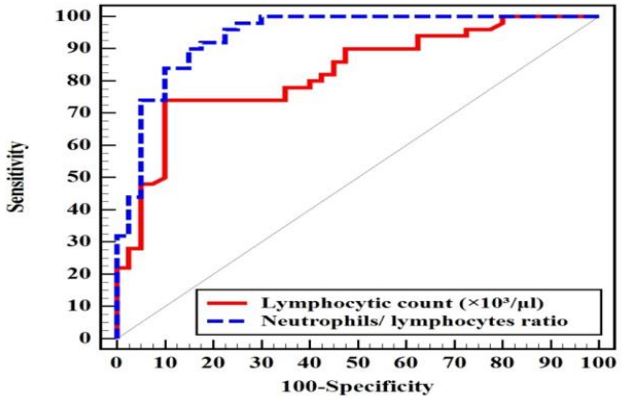

Figure (2):ROC curve for lymphocytic count and neutrophils/ lymphocytes ratio to predict ICU admission.

Based on biomarkers ROC curves, comparison to predict ICU admission was performed by analyzing diagnostic accuracy measures as displayed in Table 5 the ROC curve was used to determine an optimal cut-off value for each biomarker.

PCT had a sensitivity of 78% and a specificity of 77.5% > 0.1 ng/ml. Ferritin had a sensitivity of 94% and a specificity of 80% > 367 ng/ml. IL-6 showed a sensitivity of 96% and a specificity of 97.5% > 9.8 ng/ml, C-reactive protein had a sensitivity of 96% and a specificity of 97.5% > 39.7 mg/L.Lymphocytic count had a sensitivity of 74% and a specificity of 90% ≤1.3×10³/µl. Neutrophils/ lymphocytes ratio had a sensitivity of 90% and a specificity of 85% > 4.02 , while D-Dimer had a sensitivity of 94% and a specificity of 97% > 396 ng/ml. Lastly, total LDH had a sensitivity of 56% and a specificity of 92.5% > 440 U/L (Figure 3, 4).

Table (5): Prognostic performance for different markers to predict ICU admission.

	AUC	P	95% C.I	Cut off#	Sensitivity	Specificity	PPV	NPV
D Dimer (ng/ml)	0.978	<0.001*	0.952 – 1.000	>396	94.0	97.50	97.9	92.9
Ferritin (ng/ml)	0.911	<0.001*	0.846 – 0.975	>367	94.0	80.00	85.5	91.4
C-reactive protein (mg/L)	0.971	<0.001*	0.923 – 1.000	>39.7	96.0	97.50	98.0	95.1
Interleukin 6 (pg/ml)	0.990	<0.001*	0.973 – 1.000	>9.8	96.0	97.50	98.0	95.1
Procalcitonin (ng/ml)	0.880	<0.001*	0.814 – 0.947	>0.1	78.0	77.50	81.2	73.8
LDH (U/L)	0.754	<0.001*	0.652 – 0.855	>440	56.0	92.50	90.3	62.7
Lymphocytic count (×10³/µl)	0.824	<0.001*	0.738 – 0.910	≤1.3	74.0	90.00	90.2	73.5
Neutrophils/ lymphocytes ratio	0.940	<0.001*	0.890 – 0.989	>4.02	90.0	85.00	88.2	87.2

AUC: Area Under a Curve p value: Probability value CI: Confidence Intervals
NPV: Negative predictive value PPV: Positive predictive value
*: Statistically significant at p ≤ 0.05 #Cut off was choose according to Youden index

Table (6):Comparison between mild/moderate and severe/critical COVID-19 patients according to patients outcome.

Patients outcome	Mild/Moderate COVID-19 patients (n = 80)		Severe/ Critical COVID-19 patients (n = 100)		χ^2	P
	No.	%	No.	%		
Non-survivors patients	0	0.0	50	50.0	27.692*	<0.001*
Survivors patients	80	100.0	50	50.0		

Table (7):Comparison between survivors and non-survivors COVID-19 patients according to different parameters in all studied patients.

Parameter	Patients outcome: Non-survivors patients (n = 50)	Survivors patients (n = 130)	Test of Sig.	p
Age (years)				
Min. – Max.	34.0 – 80.0	19.0 – 75.0	t= 5.648*	<0.001*
Mean ± SD.	60.48 ± 12.28	42.57 ± 13.90		
Oxygen saturation (%)				
Min. – Max.	40.0 – 80.0	70.0 – 99.0	t= 17.211*	<0.001*
Mean ± SD.	48.20 ± 10.98	89.60 ± 9.92		
C-reactive protein (mg/L)				
IQR	48.0 – 109.0	4.20 – 48.0	U= 396.0*	<0.001*
Median (Min. – Max.)	48.0 (18.0 – 151.0)	20.10 (0.70 – 96.0)		
Ferritin (ng/ml)				
IQR	610.0 – 760.0	138.0 – 513.0	U= 253.50*	<0.001*
Median (Min. – Max.)	690.0 (352.0 – 2000.0)	357.0 (8.0 – 2127.0)		
Procalcitonin (ng/ml)				
IQR	0.20 – 0.41	0.05 – 0.20	U= 419.0*	<0.001*
Median (Min. – Max.)	0.30 (0.05 – 1.60)	0.10 (0.01 – 0.60)		
Interleukin-6 (pg/ml)				
IQR	49.0 – 101.0	2.40 – 20.0	U= 203.0*	<0.001*
Median (Min. – Max.)	61.0 (26.0 – 310.0)	4.80 (1.10 – 210.0)		
International normalized ratio				
IQR	1.05 – 1.20	1.02 – 1.03	U= 158.50*	<0.001*
Median (Min. – Max.)	1.13 (1.02 – 1.70)	1.02 (1.01 – 1.90)		

D-dimer (ng/ml)				
IQR	1500.0 – 2000.0	162.0 – 800.0	U= 128.0*	<0.001*
Median (Min. – Max.)	2000.0 (800.0 – 2000.0)	224.0 (88.0 – 2300.0)		
White blood cells (×10³/µl)				
IQR	9.96 – 16.30	5.23 – 8.45	U= 238.50*	<0.001*
Median (Min. – Max.)	12.50 (7.75 – 25.40)	6.90 (3.39 – 27.20)		
Lymphocytes (×10³/µl)				
IQR	0.48 – 0.98	1.10 – 2.42	U= 305.0*	<0.001*
Median (Min. – Max.)	0.69 (0.20 – 2.30)	1.63 (0.27 – 5.72)		
Neutrophils (×10³/µl)				
IQR	9.19 – 14.30	2.57 – 7.0	U= 212.0*	<0.001*
Median (Min. – Max.)	11.20 (5.46 – 24.70)	4.45 (0.97 – 24.40)		
Neutrophils/ lymphocytes ratio				
IQR	8.64 – 23.68	1.27 – 6.86	U= 149.0*	<0.001*
Median (Min. – Max.)	15.33 (4.46 – 123.5)	2.92 (0.26 – 19.0)		

The AUC-ROC curves were used to compare the diagnostic potential of different biomarkers including PCT, D-Dimer, IL-6, CRP, Ferritin and LDH, lymphocytic count and neutrophils/lymphocytes ratio to predict COVID-19 mortality, respectively (Figures 3 and 4). Accordingly, serum LDH had the best power to predict mortality followed by D-Dimer, and neutrophils/ lymphocytes ratio.

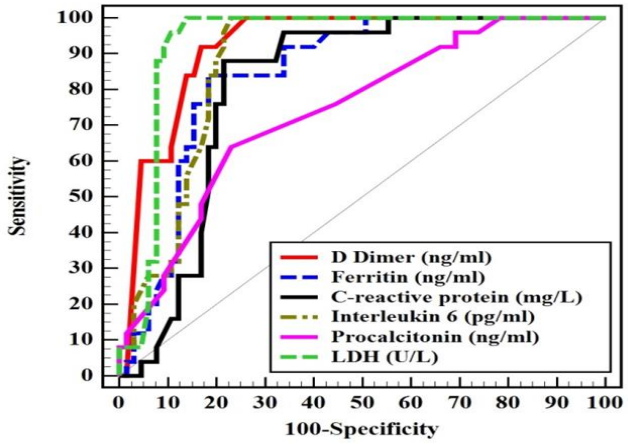

Figure (3): ROC curve for different markers to predict mortality.

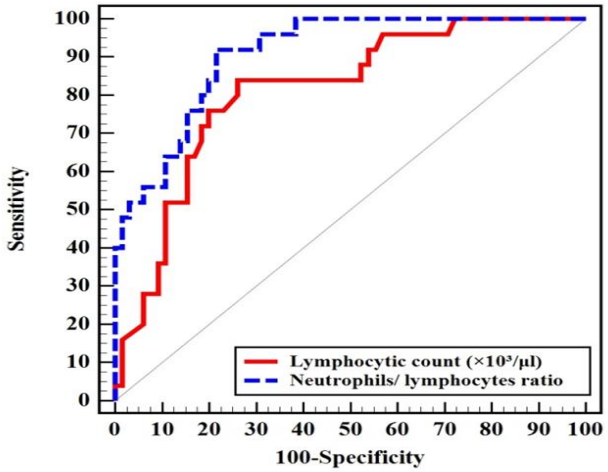

Figure (4):ROC curve for lymphocytic count and neutrophils/ lymphocytes ratio to predict mortality.

Based on biomarkers ROC curves, comparison to predict mortality was performed by analyzing diagnostic accuracy measures as displayed in Table 8 The ROC curve was used to determine an optimal cut-off value for each biomarker.

PCT had a sensitivity of 64% and a specificity of 76.9% > 0.2 ng/ml. Ferritin had a sensitivity of 84% and a specificity of 81.5% > 538 ng/ml. IL-6 showed a sensitivity of 96% and a specificity of 78.5% > 26 ng/ml. C-reactive protein had a sensitivity of 88% and a specificity of 78.5% > 82 mg/L. Lymphocytic count (×10³/µl) had a sensitivity of 84% and a specificity of 73.9% ≤1.13×10³/µl. Neutrophils/ lymphocytes ratio had a sensitivity of 92% and a specificity of 78.5% > 7.06 .while D-Dimer had a sensitivity of 92% and a specificity of 83.1% > 996 ng/ml. Lastly, total LDH had a sensitivity of 96% and a specificity of 87.7% > 430 U/L (Figure 3, 4).

Table (8): Prognostic performance for different markers to predict mortality .

	AUC	p	95% C.I	Cut off#	Sensitivity	Specificity	PPV	NPV
D Dimer (ng/ml)	0.921	<0.001*	0.867 – 0.976	>996	92.0	83.08	67.6	96.4
Ferritin (ng/ml)	0.844	<0.001*	0.763 – 0.925	>538	84.0	81.54	63.6	93.0
C-reactive protein (mg/L)	0.811	<0.001*	0.722 – 0.900	>82	88.0	78.46	61.1	94.4
Interleukin 6 (pg/ml)	0.875	<0.001*	0.805 – 0.946	>26	96.0	78.46	63.2	98.1
Procalcitonin (ng/ml)	0.742	<0.001*	0.633 – 0.852	>0.2	64.0	76.92	51.6	84.7
LDH (U/L)	0.930	<0.001*	0.873 – 0.987	>430	96.0	87.69	75.0	98.3
Lymphocytic count (×10³/µl)	0.812	<0.001*	0.717 – 0.907	≤1.13	84.0	73.85	55.3	92.3
Neutrophils/ lymphocytes ratio	0.908	<0.001*	0.849 – 0.967	>7.06	92.0	78.46	62.2	96.2

AUC: Area Under a Curve p value: Probability value CI: Confidence Intervals
NPV: Negative predictive value PPV: Positive predictive value
*: Statistically significant at $p \leq 0.05$ #Cut off was choose according to Youden index

Discussion

This study was conducted to evaluate the utility of routinely available biochemical markers in the management of COVID-19. Patients infected with SARS-CoV-2 show a higher risk of ARDS, which necessitates early detection and continuous monitoring from the initial phase to prevent adverse outcomes.

Our study aimed at finding the role of biomarkers for detecting COVID-19 severity and predicting disease outcome. We compared various biochemical and hematological biomarkers tests among groups of the current study population regarding disease severity and mortality.

The present study revealed no significant difference between mild/moderate patients and severe/critical COVID-19 patients regarding gender, whereas the mean of age was statistically significantly higher among severe /critical than mild/moderate COVID-19 patients group. In accordance with the present study, Qin, Zhou [8] reported no gender difference between severe and non-severe COVID-19 patients, while severe patients were significantly older than non-severe COVID-19 patients.

Aging is a risk factor for poor outcome in COVID-19 patients which can be attributed to gradual diminution of cilia and ciliated cells in respiratory tract leading to decrease clearance of SARS-CoV-2 particles, in addition to the disruption of innate and adaptive responses as well as the continual pro-inflammatory cytokines production in elderly that could potentially trigger inflammatory pathogenesis [9].

In the present study, the median values of serum aspartate and alanine aminotransferasesas well as LDH activities (U/L) were significantly higher in severe/critical when compared to mild/moderate COVID-19 patients. In accordance with this study, Chen, Wu [10] reported significantly higher alanine aminotransferase and LDH activities in severe patients than moderate COVID-19 patients.

The SARS-CoV-2 infection associated elevation of liver enzymes activities may be attributed to immune system injury, systemic inflammatory response syndrome and a stormy release of cytokines that can induce liver injury as well as drug hepatotoxicity rather than direct viral cytopathic effect due to higher expression of ACE2 in cholangiocytes. Moreover, COVID-19 infection may result in deterioration of previously existed chronic liver disease,which increases mortality [11]. Additionally, the increased LDH activity reflects tissue and cell destruction and it is usually associated with liver and lung diseases. Li et al.[12] assessed the impact of serum LDH levels at admission and identified it as an independent risk factor for both severity and mortality in COVID-19 cases. Acute hypoxia or inflammation caused by lung infection can lead to thrombogenesis and organ injury, highlighting LDH as a critical marker for assessing disease severity and predicting mortality in COVID-19 cases.

Eight candidate biomarkers including ferritin, PCT, IL-6, LDH , D-Dimer, CRP, lymphocytic count and neutrophils/lymphocytes ratio were chosen for comparison to to evaluate their predictive power for severity and mortality in COVID-19 infection.

Serum IL-6 , D-Dimer, and CRP had a higher AUC-ROC curve for predicting ICU admission as compared with other biomarkers. While serum LDH, D-Dimer, and neutrophils/ lymphocytes ratio had a higher AUC-ROC curve for predicting mortality as compared with other biomarker.

As regard to the inflammatory markers studied in the current work, a significantly higher median values of serum levels of ferritin, PCT, interleukin-6 (IL-6) and C-reactive protein were observed in severe/critical patients when compared to mild/moderate COVID-19 patients group. In accordance with the current work, a study reported significantly higher levels of PCT, ferritin, IL-6 and CRP in patients with severe COVID-19 presentation when compared to the non-severe [8]. Mahat, Panda [13], reported significantly higher CRP, PCT, IL-6 and ferritin levels in severe than non-severe COVID-19 patients

The SARS-CoV-2 infection can induce uncontrolled production of chemokines and cytokines, including IL-6 which is a pleiotropic pro-inflammatory cytokine with a central role in regulation of immunological and inflammatory responses. Aberrant higher production of pro-inflammatory cytokines or chemokines can result in excess tissue damage leading to respiratory failure resulting in multiorgan dysfunction in viral infections mimicking characteristics of secondary hemophagocytic lymphohistiocytosis (HLH) [14].

The elevated IL-6 level also induces multiple proteins of acute-phase including C-reactive protein, ferritin, amyloid protein and fibrinogen as well as components of complement system resulting in augmentation of inflammatory reactions and activation of coagulation pathway that lead to disruption of procoagulant–anticoagulant homeostasis, disseminated intravascular coagulation induction and multi-organ failure. IL-6 plays a crucial role in the host defense in SARS-CoV-2 infection. However, excessive secretion of IL-6 can trigger a cytokine storm, resulting in a severe systemic inflammatory response. Utilizing of humanized anti-IL-6 receptor antibody, tocilizumab (IL-6 blockade therapy) has shown effectiveness in treating COVID-19 infections [(15)].

Serum ferritin, an indicator of iron storage, is known to rise in conditions such as inflammation, hepatic diseaes and malignancies. In COVID-19 patients, serum ferritin levels have been observed to increase significantly, primarily due to the cytokine storm associated with severe infection. Ferritin plays a crucial role in protecting the body against active infection by regulating the availability of iron to pathogens, thereby limiting their growth and proliferation [(16)]. Elevated ferritin levels can activate endothelial cells in pulmonary vessels, disrupting normal hemostasis, fibrinolysis regulation, and vascular permeability. This imbalance contributes to the development of COVID-19-associated vasculopathy driven by inflammation[(17)].

Procalcitonin is a peptide precursor of calcitonin. Increased PCT level can occur in sepsis and septic shock. During bacterial infection, amplified maintained production of procalcitonin by elevated of IL-6, IL-1β, and TNF-α whereas increased IFNγ in viral infection inhibits PCT synthesis. Therefore, the level of PCT in the majority of non-severe COVID-19 patients, remains within normal interval and elevated level may indicate secondary bacterial infection in severe COVID-19 patients, which could increase the probabilities of fatal outcome [(18)].

COVID-19 associated hyperinflammation leads to elevated levels of D-Dimer and fibrinogen, resulting in hypercoagulation and complications such as Disseminated Intravascular Coagulation (DIC) and multi-organ dysfunction. D-Dimer levels are significantly higher in patients with severe infections compared to those with milder forms of the disease. Elevated D-dimer levels indicate thrombosis, and increased Fibrin Degradation Products (FDP) are a result of thrombolysis. The administration of anticoagulant therapy, particularly low molecular weight heparin, has been associated with improved prognosis by reducing the occurrence of venous thromboembolism and Disseminated Intravascular Coagulation [(19)].

In the present study, the coagulation status showed significantly higher median of plasma levels of INR and D-dimer in severe /critical patients than mild/moderate COVID-19 patients, whereas the mean value of platelets count was statistically significantly lower in severe/critical when compared with mild/moderate COVID-19 patients. In accordance with this study,Yu, Qin (20), reported that patients with severe COVID-19 illness showed higher prothrombin time and D-Dimer levels than mild patients. Another study byLippi, Plebani [(21)], reported that, low platelets count showed an association with an elevated risk of COVID-19 severity.

Increased INR, D-Dimer levels and low platelets count in COVID-19 cases can be attributed to direct SARS-CoV-2 infection of bone marrow cells resulting in inhibition of platelet synthesis, induction of platelet destruction due to increased levels of autoantibodies in COVID-19 and increased platelets aggregation in the lungs producing microthrombi in addition to cytokines storm that can increase D-dimer and prolong prothrombin time [(22)]. Serum D-Dimer had the best power to predict mortality following LDH activiy.

The total and differential white blood cells count in the current study revealed statistically significantly higher median values of total white blood cells and neutrophils counts of severe/critical than mild/moderate COVID-19 patients, while the median value of lymphocytes count was significantly lower in severe/critical patients than mild/moderate COVID-19 patients.

The median value of neutrophils/lymphocytes ratio was statistically significantly higher in severe/critical patients group than mild/moderate COVID-19 patients. These findings were in accordance with two studies revealed significantly higher white blood cells and neutrophils counts in severe COVID-19 patients compared to non-severe, whereas lymphocytes count was significantly lower in severe COVID-19 patients than non-severe [(8, 23)]. Mahat, Panda [(13)], reported that severeCOVID-19 patients showed significantly higher neutrophils/lymphocytes ratio than non-severe patients. Li, Liu (24) reported significantly higher level of neutrophils/lymphocytes ratio in severe COVID-19 patients than mild patients.

It was found that, severe or non-survivors patients with COVID-19 showed progressive reduction of lymphocytes count while neutrophils count showed a gradual increase. Neutrophils act as pro-inflammatory cells induced by virus-related inflammatory mediators such as IL-6 and IL-8. Therefore, neutrophils/lymphocytes ratio increases with severe COVID-19 infection [(25)].

Lymphopenia can enhance viral replication, delay viral elimination, shift the adaptive immune response to innate immune response, increase activation of macrophages and neutrophils, induce uncontrolled production of cytokines, which finally result in multi-organ failure [(3)]. In COVID-19 patients, lymphopenia with elevated neutrophils/lymphocytes ratio may be attributed to different mechanisms affecting lymphocytes production or survival.

It was suggested that SARS-CoV-2 can infect haematopoietic stem cell expressing ACE2 resulting in pyroptosis of these cells that may contribute to reduced lymphocytic production [(26)]. The SARS-CoV-2 may also infect primary lymphoid organs, in addition to loss, degeneration and necrosis of lymphocytes in spleen that may aid in the reduction of lymphocytes count [(27)]. SARS-CoV-2 may infect lymphocytes directly. CD8+ cytotoxic T lymphocyte may act to eliminate the infected lymphocytes through Fas- FasL interaction as well as perforin and granzymes release.$CD4^+$ T cells were found to be the major constituent of alveoli and

other organs inflammatory infiltrates, resulting in sequestration of lymphocytes in lung as well as probably other infected organs [3].

Additionally, the SARS-COV-2 induced apoptosis was suggested to occur due to key pro-apoptosis gene higher expression, p53, that was assessed in peripheral blood mononuclear cell of COVID-19 patients. Moreover, the production of TNF-α by SARS-CoV-2 infected macrophages results in apoptosis of T lymphocytes [28].

The IL-6 was reported to suppress lymphopoiesis by direct effects on haematopoietic stem or progenitor cells. Tocilizumab (monoclonal antibody against IL-6) treatment was found to improve lymphocytes count [29]. Additionally, patients with severe COVID-19 presentation showed elevated levels of antiviral IgG and IgM, in addition to marked lymphopenia compared with mild/moderate patients, suggesting that anti-SARS-CoV-2 antibodies may promote lymphopenia pathogenesis. Systemic autoimmune rheumatic disease related autoantibodies were also reported to be produced in patients with severe COVID-19 presentation without previous underlying autoimmune disease [30].

In the present study, upon classification of studied patients according to outcome, severe /critical COVID-19 patients showed a higher mortality frequency than mild/moderate COVID-19 patients. A study by Weiss and Murdoch [31], revealed that severe or critical COVID-19 illness showed high mortality. Another study observed that critical COVID-19 patients showed an increased mortality outcome [32].

In this study, upon comparing survivors and non-survivors COVID-19 patients according to inflammatory markers, non-survivors showed significantly higher median values of serum C-reactive protein, ferritin, procalcitonin and interleukin 6 levels than survivors. In accordance with the current work, Mahat, Panda [13], reported significantly higher CRP, procalcitonin, interleukin 6 and ferritin in non-survivors than survivors COVID-19 patients.

In the current study, non-survivors COVID-19 patients showed significantly higher median values of plasma levels of international normalized ratio and D-Dimer than survivorsCOVID-19 patients. This finding was in agreement with a preceding study that reported a significantly higher D-dimer level in non-survivors than survivorsCOVID-19 patients [33].

In the present work, non-survivors COVID-19 patients showed significantly higher median values of white blood cells and neutrophils counts as well as neutrophils/ lymphocytes ratio than survivorsCOVID-19 patients, while , the median value of lymphocytes count was statistically significant lower in non-survivors than survivorsCOVID-19 patients.

In accordance with the current study, Mahat, Panda [13], reported significantly higher levels of neutrophils/lymphocytes ratio in COVID-19 non-survivors than survivors. Another study reported that, increased neutrophils count and neutrophils/lymphocytes ratio with decreased lymphocytes count have been associated with worse clinical outcome of COVID-19 infection [34].

Conclusion

Our observations highlight the efficacy of several biochemical markers. Biomarkers were significantly different amongst study population groups. Patients with ICU admission revealed highest concentrations. Based on the AUC-ROC analysis, serum IL-6 demonstrated the highest predictive power for the need of ICU admission followed by D-Dimer, and CRP, while mortality was most strongly indicated by serum LDH followed by D-Dimer, Neutrophils/ lymphocytes ratio, IL-6 and ferritin.

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