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Neutrophil to Lymphocyte Ratio (NLR) and Derived NLR Combination: A Cost-effective Predictor of Moderate to Severe COVID-19 Progression

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ABSTRACT

Inflammation is an essential contributor to Coronavirus disease 2019 (COVID-19). In this regard, finding a prognostic indicator is valuable because the treatment will be more effective if critical patients with high inflammation are diagnosed earlier. We aimed to evaluate some hematologic markers for COVID-19 and assess their association with the severity of the disease.

A total of 154 COVID-19 patients were laboratory-confirmed and admitted to Imam Khomeini Hospital Complex, Tehran, Iran, from February 12, 2020, to April 4, 2020, and 55 healthy individuals were enrolled in the study. The severity of the patients' illnesses was classified into three subgroups according to the types of oxygen therapies (moderate (61), severe (28), and critical (43)) and examined the different ratios of total white blood cell (WBC) count, neutrophil to lymphocyte ratio (NLR), platelet to monocyte ratio (PLR), macrophage to lymphocyte ratio (MLR), derived NLR ratio (dNLR), and some biochemical tests.

COVID-19 patients had higher levels of NLR, MLR, PLR, and dNLR than healthy subjects. receiver operating characteristic (ROC) analysis of the curve revealed that NLR and dNLR had a high diagnostic value to differentiate COVID-19 patients from healthy subjects (area under the curve [AUC]=0.923 and 0.910, respectively) and predict mortality (AUC=0.726 and 0.735, respectively).

NLR and dNLR may be reliable markers to evaluate the severity of COVID-19. NLR and dNLR had a high diagnostic value for differentiating COVID-19 patients from healthy subjects, and they could predict the severity and outcome of the disease.

Keywords: COVID-19; Inflammation; Prognosis; SARS-CoV-2

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INTRODUCTION

In early 2020, the new outbreak of coronavirus outbreak, Coronavirus disease 2019 (COVID-19),

reached a pandemic after originating in Wuhan, China.[1] The clinical characterization of COVID-19 patients can be mild or severe. Mild and moderate cases have mild symptoms with a good prognosis, but severe patients progressed rapidly to the acute phase and underwent respiratory failure with a high mortality rate.[1] Based on the World Health Organization (WHO) guidelines, COVID-19 disease severity is classified as follows: mild disease, symptomatic patients with no signs of viral pneumonia or hypoxia; moderate disease, patients with clinical symptoms of pneumonia (fever, cough, dyspnea, fast breathing) but no sign of severe pneumonia, including $SpO_2 \geq 90\%$; and serious disease, patients with clinical signs of pneumonia (fever, cough, dyspnea, fast breathing) plus one of the following symptoms of respiratory rate>30 breaths/min, severe respiratory distress, or SpO2<90% in room air. Severe respiratory distress syndrome (ARDS), septicemia, and septic shock are critical conditions.[2]

The pathophysiology of COVID-2 is not completely clear, and the puzzle pieces are not fully put together yet. As we know, inflammation is a critical component of infectious diseases. Some research confirmed that inflammation is a factor in the development of viral pneumonia.[3] On the other hand, pro-inflammatory cytokines increased in the sera of some SARS-CoV-2.[4]

Besides C-reactive protein (CRP), the number of neutrophils and lymphocytes, the number of white blood cells (WBC) count, the neutrophil to lymphocyte ratio (NLR), besides the percentage of platelet to lymphocyte (PLR) are markers of systemic inflammation.[5,6]

Late detection and inappropriate management are important causes of increased mortality of this infection. Appropriate clinical indicators to identify and differentiate severe patients early can facilitate medical intervention and reduce mortality.[7,8,9] Inflammation is a crucial contributor to the development of COVID-19 and influences its prognosis and symptom. NLR is a systemic inflammation indicator that can be measured during routine hematology.[5] Many abnormal parameters in COVID-19 patients are found, such as high levels of D-dimer, erythrocyte sedimentation rate (ESR), CRP, lactate dehydrogenase (LDH), ferritin, and interleukin (IL)-6.[10,11] However, NLR is reliable, easy-to-access, and cost-effective biomarker that has been used as a prognostic indicator in many studies, including sepsis, cardiovascular, and malignant diseases.[9,12-14]

On the other hand, NLR exhibits the highest specificity and sensitivity for the severity of disease compared to CRP, MLR, and PLR.[15] Inflammatory tests such as D-dimer, CRP, LDH, and IL6 compared to NLR are more time-consuming, costlier. They may not be available in every lab in low-income areas. Besides, the sensitivity and quality of the test depend on the test conditions.

Hence, biomarkers representing inflammation are potential predictors for predicting severe cases of COVID-19 patients.[6] Total white blood cell (WBC) count, NLR, PLR, MLR, derived NLR ratio (dNLR), neutrophil count divided by the result of WBC count minus neutrophil count, are indicators of inflammation[6] that are presented as prognosis predictors for patients with viral pneumonia.[16] Many studies have used NLR to predict the seriousness of COVID-19 disease. Still, there is heterogeneity between the cut-offs and the association with other inflammatory factors due to differences in the study population. This study should be done on different people for definitive use and determining cut-offs to be more accurate. This study examined NLR, MLR, PLR, and dNLR, and their association with other inflammatory factors was also assessed. We attempted to identify the association of NLR, MLR, PLR, and dNLR with the seriousness and outcome of COVID-19 infection. Finally, we tried to select the best biomarker as a prognostic marker in COVID-19 disease.

MATERIALS AND METHODS

Study Population and Clinical Evaluation

Inpatients suspected of COVID-19 admitted to Imam Khomeini Hospital Complex, Tehran City, Iran, were enrolled from February 12 to April 4, 2020. They were diagnosed by the interim guidance of the WHO[17] and detailed national guidelines for diagnosing and treating COVID-19 (the sixth version). Patients were followed up until May 5, 2020, when the outcome for all patients was established. The study was approved by the National Ethics Committee on Research in Medical Sciences of the Iranian Ministry of Health (Ethical Code: IR.NIMAD.REC.1398.411).

Concerning guidelines, all patients were provided with a nasopharyngeal swab for COVID-19 RT-PCR (real-time polymerase chain reaction) and acquired a chest computed tomography (CT) scan. Demographics data, important dates during the process, clinical features, and radiological findings were collected from

the patient's medical records by a clinician and rechecked with another. The patients without PCR results and those with tumors or pregnant ones were excluded. Finally, 154 confirmed cases with COVID-19 and 55 healthy individuals were enrolled in the study.

The clinical status of the patients was recorded. All patients did not receive any treatment before blood sampling. A complete blood count (CBC) was taken, and the hematology findings were assessed.

The severity of the Patient was categorized into three subsets based on the types of oxygen therapies. The patients with a supportive O2 nasal cannula or masks were considered moderate (no evidence of severe pneumonia). Those who were hospitalized in the intensive care unit (ICU) and were provided noninvasive ventilation (NIV) masks were categorized in the influential group (SpO2<90%;), and those who needed ICU and mechanical ventilators (intubated) were classified as critical patients (respiratory failure).[18] Based on the patients' conditions, was obtained in writing or orally.

Blood Sample

Peripheral venous blood samples were assessed at Clinical Simorgh Laboratory following standard operative procedures. The separation and preparation of the blood samples were performed following the safety procedure. The serums were isolated after clotting, centrifuged at 3000 rpm for 15 minutes at room temperature, then freshly used for biochemical analysis. Furthermore, the samples were aliquoted, labeled, and frozen at -80°C to assess other relevant molecules.

A complete blood count was carried out using Automated Sysmex (XS 500i full diff, Japan). NLR, PLR, MLR, and dNLR are calculated as follows: NLR: divides the neutrophils into the lymphocytes, PLR divides the platelet count into lymphocyte count, MLR: divide monocyte into the lymphocytes, dNLR: divide the neutrophils into the WBC (White Blood Cell) Count- neutrophils count. Serum biochemical tests, for instance, fasting blood sugar (117500, Pars Azmun, Iran), LDH (DDP01182-S, Delta. DP, Iran), and CRP (3040, BIONIK DIAGNOSTIC SYSTEMS, Iran) were also assessed by Hitachi-91 auto-analyzer (Japan). The serum levels of troponin I (VIDAS TNHS) were measured using VIDAS bioMerieux, (France), and serum levels of D-dimer (L2KDD2) and ferritin (L2KFE2) were assessed by an automated immunoassay (IMMULITE 2000, Siemens Healthineers, the United Kingdom). Serum levels of IL-6 were evaluated by the IMMULITE 2000 Immunoassay System (ere assessed using an automated immunoassay (IMMULITE 2000 Immunoassay System, Siemens Healthcare Diagnostics Inc., The United States of America).[19,20]

Statistical Analysis

Analyzing data was done; using SPSS 19.01 statistical software. The hematological cell count data are expressed as 10^9/L. The non-normal distribution data was represented by the median (quartile value). The mean values were compared between the groups using the analysis of variance (ANOVA) test. Binary logistic regression analysis was used to incorporate NLR and other laboratory markers. The efficiency of diagnosing patients' disease stages was analyzed using the receiver operating characteristic (ROC) curve. The laboratory findings were as mean±SD or median, and a comparison between groups was made; using the Mann-Whitney test. The correlation of laboratory parameters and the relationship between mortality related to laboratory findings was calculated with the Spearman rank correlation coefficient. A *p-value* of less than 0.05 was considered significant.

RESULTS

Demographics Characteristics

A total of 154 confirmed patients with SARS-CoV-2 infection and 55 age- and sex-matched healthy subjects were enrolled in the study. A total of 108 patients (56.54%) were male, and 83 (43.45%) were female. COVID-19 patients with a higher BMI, more than>30 kg/m2, are associated with hospitalization compared to the control; group (Table 1).

Association of NLR, MLR, PLR, and dNLR with Disease Severity and Mortality in COVID-19 Patients

NLR, MLR, PLR, and dNLR levels were significantly higher in COVID-19 patients. We also compared these factors in alive and expired COVID-19 patients. The NLR, MLR, and dNLR levels were considerably higher in the deceased patients compared to survived COVID-19 patients. The difference in PLR values was not significant (Table 2).

Table 1. Demographic characteristics of study groups

Indicators	COVİD-19 group n=154	Control group n=55	*p*
Weight (Mean±SD)	82±14	79±14	0.199
Age (Mean±SD)	59.6±14.7	57.4±8.7	0.318
BMI <30	68.9%	85.4%	**0.044**
>30	31.1%	14.6%	

Table 2. The comparisons of the neutrophil to lymphocyte ratio (NLR), macrophage to lymphocyte ratio (MLR), platelet to monocyte ratio (PLR), and dNLR between study groups (COVID-19 patient and control; COVID-19 Alive and COVID-19 expire)

	N	Mean±SD	Median	*p*
		NLR		
COVID-19	154	13.28±20.47	7.56	**<0.001**
Control	55	1.76±0.68	1.61	
COVID-19 Alive	110	8.58±7.39	5.80	**<0.001**
COVID-19 Expire	44	25.03±33.97	13.76	
		MLR		
COVID-19	136	0.73±0.59	0.57	**<0.001**
Control	55	0.26±0.08	0.25	
COVID-19 Alive	95	0.62±0.35	0.55	**0.021**
COVID-19 Expire	41	1.00±0.88	0.76	
		PLR		
COVID-19	136	403.86±330.22	273.49	**<0.001**
Control	55	110.11±44.98	113.04	
COVID-19 Alive	95	380.89±308.54	256.60	0.292
COVID-19 Expire	41	457.08±374.40	372.34	
		dNLR		
COVID-19	154	6.34±6.36	4.63	**<0.001**
Control	55	1.28±0.42	1.20	
COVID-19 Alive	110	4.84±3.76	3.34	**<0.001**
COVID-19 Expire	44	10.08±9.40	7.58	

p: Comparison between Groups

To examine the relationship between NLR, MLR, PLR, and dNLR and disease severity, we divided them into three groups (moderate, severe, and critical). We examined the ratios of NLR, MLR, PLR, and dNLR in these groups. In this study, 154 patients with COVID-19 infection, 61 were diagnosed as moderate, 28 cases as severe, and 43 points as critical on admission (Table 3).

Table 3. Association of neutrophil to lymphocyte ratio (NLR), macrophage to lymphocyte ratio (MLR), platelet to monocyte ratio (PLR), and dNLR with disease severity in COVID-19 patients

	N	Mean±SD	Median	*p*	
		NLR			
Critical	43	22.49±25.98	13.10		
Severe	28	9.59±4.64	8.47	**0.004**[1]	
Moderate	61	7.35±7.44	3.76	**<0.001**[2]	**0.001**[3]
		MLR			
Critical	43	0.93±0.82	0.73		
Severe	23	0.62±0.34	0.54	0.168[1]	
Moderate	57	0.64±0.46	0.54	0.039[2]	0.827[3]
		PLR			
Critical	43	481.88±406.88	373.08		
Severe	23	401.50±300.20	255.56	0.742[1]	
Moderate	57	372.80±301.36	231.30	0.282[2]	0.398[3]
		dNLR			
Critical	43	9.61±6.87	7.88		
Severe	28	5.92±3.04	5.33	**0.014**[1]	
Moderate	61	3.88±3.68	2.48	**<0.001**[2]	**<0.001**[3]

p[1]: Comparison between Critical and Severe. (Mann-Whitney)

p[2]: Comparison between Critical and Moderate. (Mann-Whitney)

p[3]: Comparison between Severe and Moderate. (Mann-Whitney)

For the moderate group, the NLR (median value 3.76) and dNLR (median value 2.48) were significantly lower than those of the severe and critical groups. Also, in the severe group, the NLR (median value 8.47) and dNLR (median value 5.33) were significantly lower than those in the critical group (NLR median value of 13.10 and dNLR median value of 7.88) ($p<0.05$). There was no significant difference between the moderate, severe, and critical groups in PLR or MLR ($p>0.05$) (Table 3).

CBC Results Using the Optimum Cut-off Values

To explore risk factors that can predict the prognosis of individuals with COVID-19, we used ROC analysis. Because no laboratory reference values were found for NLR, PLR, MLR, and d NLR, we analyzed the optimal cut-off values computed by the ROC analysis. The ROC of NLR, PLR, MLR, and dNLR in predicting the COVID-19 infection and the severe prognosis are shown in Figures 1 A and B. The areas under the curve (AUC) of NLR, PLR, MLR and dNLR in predicting COVID-19 infection were 0.923, 0.904, 0.890, 0.910 with cut-off values of 3.162, 172.515, 0.303 and 2.260, respectively (Figure 1C). The highest specificity and sensitivity were as follows: 0.964 and 0.818 for NLR, 0.982 and 0.794 for PLR, 0.964 and 0.799 for d-NLR.

Additionally, the AUCs of NLR, PLR, MLR, and dNLR in predicting death outcome were 0.726, 0.557, 0.625 and 0.731 with cut-off values of 6.830, 363.845,

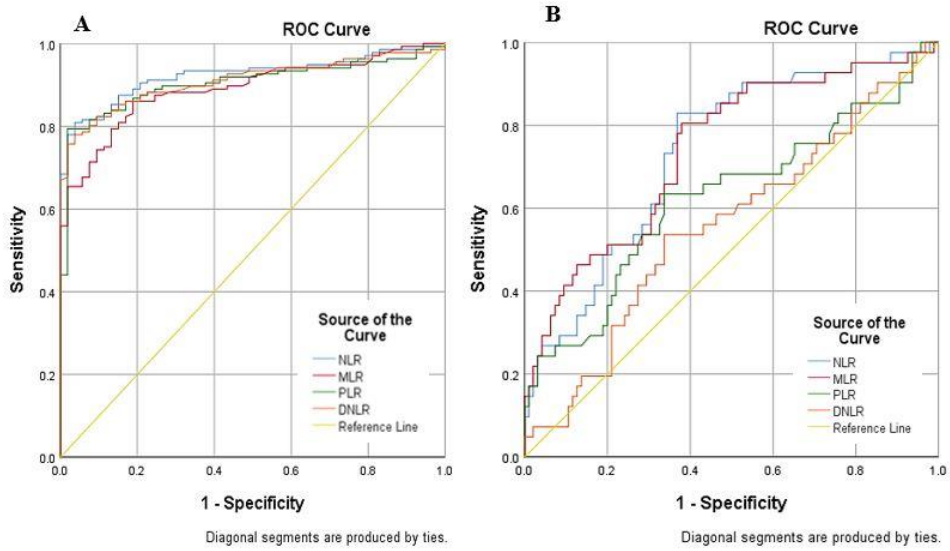
0.639 and 4.115, respectively (Figure 1C). The highest specificity and sensitivity were as follows: 0.562 and 0.828 for NLR, 0.573 and 0.841 for d-NLR, and 0.964 and 0.799 for MLR, respectively.

PLR is not appropriate as a potential diagnostic biomarker to predict death outcomes because of its AUC and P-value. In summary, we selected NLR and dNLR for further assessments.

Association of NLR and dNLR Cut-off Values with the Risk of COVID-19 Progression

In the next step, the patients were divided into three groups according to NLR cut off points (<3, 3-10, and>10), two groups according to MLR cut-off points (<0.3 and >0.3), two groups according to PLR cut off points (<172.5 and >172.5), and two groups according to dNLR cut off points (<2.26 and >2.26).

A statistically significant difference was determined concerning the NLR ratio of the three groups (Table 4). More interestingly, an NLR >10 was found in the critical group with COVID-19 (65.1%) and decreased by disease severity in severe and moderate groups (42.9% and 24.6%, respectively) (Table 4). Conversely, the low ratio of NLR (NLR<3) was seen more in the moderate (36.1 %) rather than severe and critical groups (0 % vs. 2.3%, respectively) (Table 4).



C ROC curve analysis for diagnostic value of hematologic markers for COVID-19

Control vs COVID-19	AUC	95%CI	p-value	optimal cutoff value	Specificity (%)	Sensitivity (%)
NLR	0.923	0.885-0.961	**<0.001**	3.162	96.4	81.8
PLR	0.904	0.859-0.948	**<0.001**	172.515	98.2	79.4
MLR	0.890	0.845-0.935	**<0.001**	0.303	80.0	86.0
dNLR	0.910	0.869-0.950	**<0.001**	2.260	96.4	79.9
COVID-19 alive vs expire patient						
NLR	0.726	0.674-0.806	**<0.001**	6.830	56.2	82.8
PLR	0.557	0.450-0.664	0.292	363.845	66.3	53.7
MLR	0.625	0.515-0.735	**0.021**	0.639	66.3	63.4
dNLR	0.731	0.643-0.818	**<0.001**	4.115	57.3	84.1

Figure 1. The receiver operational characteristics (ROC) curve was conducted to assess the diagnostic value of hematologic markers for COVID-19. Diagnostic value of neutrophil to lymphocyte ratio (NLR), platelet to monocyte ratio (PLR), macrophage to lymphocyte ratio (MLR), and derived NLR ratio (dNLR) in A) differentiating COVID-19 patients from control (healthy Subjects) and B) determining COVID-19 patients alive from expired; C, ROC curve analysis was conducted to assess the diagnostic value of hematological markers for COVID-19.

Table 4. Comparison of different scores of neutrophil to lymphocyte ratio (NLR), macrophage to lymphocyte ratio (MLR), platelet to monocyte ratio (PLR), and derived NLR ratio (dNLR) between COVID-19 patients with different disease severity.

		Critical N (%)	Severe N (%)	Moderate N (%)	*p*
NLR	<3	1 (2.3)	0 (0)	22 (36.1)	**<0.001**
	3-10	14 (32.6)	16 (57.1)	24 (39.3)	
	>10	28 (65.1)	12 (42.9)	15 (24.6)	
p		0.102[1]	0.102[2]	**<0.001[3]**	
MLR	<0.3	5 (11.6)	4 (17.4)	9 (15.8)	0.774
	>0.3	38 (88.4)	19 (82.6)	48 (84.2)	
p		0.5161	0.862	0.5533	
PLR	<172.5	10 (23.3)	4 (17.4)	13 (22.8)	0.841
	>172.5	33 (76.7)	19 (82.6)	44 (77.2)	
p		0.579[1]	0.592[2]	0.958[3]	
dNLR	<2.26	2 (4.7)	0 (0)	25 (41.0)	**<0.001**
	>2.26	41 (95.3)	28 (100)	36 (59.0)	
p		0.247[1]	**<0.001[2]**	**<0.001[3]**	

p value[1]: Comparison between Critical and Severe

p value[2]: Comparison between Severe and Moderate

p value[3]: Comparison between Critical and Moderate

p-value: Comparison between Groups

Association of NLR and dNLR Cut-off Points with the COVID-19 Mortality

Our results showed that NLR (median values of 5.58 and 13.76 for survived and expired patients, respectively) and dNLR (median values of 3.34 and 7.58 for survived and expired patients, respectively) are significantly different between alive and expired groups. Hospital death increased dramatically with an elevated level of NLR and dNLR (p<0.004 and p<0.009) (Table 2). Elevated NLR exhibited an increase in the risk of mortality.

An NLR<3 was found more significantly in survived patients compared to expired patients (20.9% vs. 6.8%, respectively) (Table 5). Meanwhile, our result showed that patients with higher NLR (NLR>10) had a higher chance of death in comparison to survived patients (59.1% vs. 31.8%) (Table 5).

A similar trend was observed with dNLR. A dNLR of less than 2.26 was found more significantly in survived patients compared to expired patients (25.5% vs. 6.8%, respectively) (Table 5). Meanwhile, our result showed that patients with higher dNLR (dNLR>2.26) had a higher chance of death in comparison to survived patients (93.2 vs. 74.5%) (Table 5).

Correlation between NLR and dNLR with other Inflammatory Parameters in COVID-19 Patients

Correlation analysis showed that LDH (r=0.516, p<0.001), D-dimer (U/mL) (r=0.519, p<0.001), ferritin (r=0.310, p<0.001) were significantly correlated to NLR. These correlations were also seen with dNLR. In a similar trend, LDH (r=0.512, p<0.001), D-dimer (U/mL) (r=0.518, p<0.001), ferritin (r=0.302, p<0.001) were significantly correlated to dNLR (Table 6).

Association of NLR and dNLR with other Inflammatory Parameters in COVID-19 Patients

Besides, this study investigated the association of NLR and dNLR as inflammatory markers with other inflammatory parameters. The correlation of NLR and dNLR with different parameters, including serum LDH, Ferritin, and D-dimer, revealed a significant association; furthermore, LDH, Ferritin, D-dimer, and IL6 showed linear association (Table 7).

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Table 5. Comparison of different scores of neutrophil to lymphocyte ratio (NLR), macrophage to lymphocyte ratio (MLR), platelet to monocyte ratio (PLR), and derived NLR ratio (dNLR) between alive and expired COVID-19 patients.

		Alive N (%)	Expire N (%)	*p*
NLR	<3	23 (20.9)	3 (6.8)	**0.004**
	3-10	52 (47.3)	15 (34.1)	
	>10	35 (31.8)	26 (59.1)	
MLR	<0.3	13 (13.7)	6 (14.6)	0.883
	>0.3	82 (86.3)	35 (85.4)	
PLR	<172.5	20 (21.1)	8 (19.5)	0.838
	>172.5	75 (78.9)	33 (80.5)	
dNLR	<2.26	28 (25.5)	3 (6.8)	**0.009**
	>2.26	82 (74.5)	41(93.2)	

p-value: Comparison between Groups

Table 6. Correlation of neutrophil to lymphocyte ratio (NLR), macrophage to lymphocyte ratio (MLR), platelet to monocyte ratio (PLR), and derived NLR ratio (dNLR) with other inflammatory parameters in COVID-19 patients.

	LDH		D-Dimer (U/mL)		IL6		Ferritin	
	r	***p***	**r**	***p***	**r**	***p***	**r**	***p***
NLR	0.516	**<0.001**	0.519	**<0.001**	0.175	**0.034**	0.310	**<0.001**
PLR	0.362	**<0.001**	0.205	**0.034**	-0.026	0.769	0.193	**0.026**
MLR	0.170	0.064	0.234	**0.015**	0.065	0.453	0.240	**0.006**
DNLR	0.512	**<0.001**	0.518	**<0.001**	0.179	**0.030**	0.302	**<0.001**

Table 7. Association of neutrophil to lymphocyte ratio (NLR) and derived NLR ratio (dNLR) cut-offs with other inflammatory parameters in COVID-19 patients

[a]	NLR<3	NLR 3-10	NLR>10	*p* (ANOVA)	*p* (Linear)	dNLR<2.26	dNLR >2.26	*p*
	Mean ± SD	Mean ± SD	Mean ± SD			Mean ± SD	Mean±SD	
LDH	529.54±166.80	687.45± 243.85	878.24±328.42	**<0.001**	**<0.001**	533.55 ±157.46	787.98 ±302.81	**<0.001**
CRP	18.31±8.87	24.66±7.34	21.98 ±8.40	**0.009**	0.079	18.73 ±9.26	23.42 ±7.80	**0.011**
Ferritin	566.3±393.7	802.9±483.3	950.0 ±492.2	**0.004**	**0.001**	612.6 ±414.9	875.3 ±493.4	**0.008**
D-Dimer (U/mL)	1648.68±2891.35	3349.98±4928.12	7182.22 ±6017.02	**<0.001**	**<0.001**	1706.91 ±2653.57	5468.04 ±5896.22	**0.004**
IL6	18.50±19.16	85.13±167.61	98.03 ±199.12	0.133	**0.049**	17.20 ±17.83	94.93 ±186.25	**0.001**

DISCUSSION

Despite all efforts, restrictions, and health care facilities, COVID-19 infection has become a global health challenge. Because of its various symptoms and complications in different individuals, a cost-effective way to identify infected people and their progression to a critical state is essential. Recently, several studies have been performed to use markers for the prognosis of COVID-19. In most patients with severe COVID-19, serum levels of pro-inflammatory cytokines such as Granulocyte colony-stimulating factor (G-CSF), Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF), Interferon gamma-induced protein 10 (IP10), monocyte chemoattractant protein 1 (MCP1), Macrophage inflammatory proteins MIP1α (MIP1α) (also known as CCL3), tumor necrosis factor (TNF) and IL-6, IL-8, IL-17, IL-1β, IL-2, was significantly increased as well as a high level of C-reactive protein and D-dimer, that characterized as a cytokine storm.[21,22]

In this study, we assessed the correlation of NLR, MLR, PLR, and dNLR with the severity and mortality of COVID-19 to select the best biomarker as a feasible method for evaluating the inflammatory status of COVID-19 patients. The demographic characteristics of our study patients showed that most COVID-19 patients had a BMI value of more than 30 kg/m^2 in comparison to the control group, which is consistent with the findings of other studies.[23]

The results indicated that NLR, MLR, PLR, and dNLR values in hospitalized COVID-19 patients were significantly higher than those in the control group (7.8, 2.8, 3.6, and 4.9 fold increase, respectively) is consistent with the results of similar previous studies.[15,24-26] Our result also showed significantly higher levels of NLR, MLR, and dNLR in expired patients compared to the survived group (2.91, 1.6, and 2-fold increase, respectively). In the next step, the patients were divided into moderate, severe, and critical groups to examine the relationship between NLR, MLR, PLR, and dNLR levels and disease severity. The different NLR, MLR, PLR, and dNLR values were compared between patients in the moderate, severe, and critical groups. NLR and dNLR values were significantly different in these groups. NLR increased 3.05 and 1.3-fold in the vital and severe groups compared to the moderate group. Also, dNLR increased 2.47 and 1.52-fold in critical and severe cases compared to the moderate group. So, these ratios help us differentiate acute patients from severe/moderate cases.

A recent study, after excluding the possible effects of age and gender, showed a correlation between NLR and the severity of the disease. Meanwhile, the correlation of WBC, CRP, PLR, and d-NLR indexes with the disease severity was not uncertain. [15] It was shown that NLR is correlated to the severity of disease in COVID-19 patients aged≥60 y.[27]

The absence of an NLR value reference is a limitation that needs ROC analysis.[13] We calculated cut-off values by the ROC curve and determined the cut-off levels in the admission groups. The area under the ROC curve of the NLR and dNLR predicting the infection and mortality were the largest with the highest sensitivity and specificity to predict COVID-19 and mortality. Our results showed that the NLR yielded a relatively high AUC (0.923, 95% CI: 0.885-0.961) to indicate infection with high sensitivity. Also, dNLR delivered a relatively high AUC (0.910, 95% CI: 0.869-0.950) to predict disease with high sensitivity. Our result for differentiating alive and expired patients showed that the NLR yielded a relatively high AUC (0.726, 95% CI: 0.674-0.806) with high sensitivity. Also, dNLR delivered a relatively high AUC (0.731, 95% CI: 0.643-0.818) with high sensitivity.

After dividing patients according to the optimum cut-off points for NLR, dNLR, MLR, and PLR, our results show significant differences between NLR and dNLR groups and the severity of the disease statistically. Our study further demonstrated that critical patients with increased NLR (>10) were significantly more than severe and moderate patients. There was a 22% and 41% increase compared to severe and moderate patients. The NLR>2.26 had an increase of 35% compared with the moderate patient. In a meta-analysis regarding NLR predictive value for COVID-19 patient severity, a different cut of NLR was reported in further research.[28]

NLR and dNLR distinguished the critical COVID-19 cases from the severe/moderate cases (p<0.05). Our results showed that increased NLR and dNLR correlated with the disease's severity. However, the lowest NLR and dNLR ratios (NLR<3.5 and dNLR <2.26) were 33 % and 36% lower than moderate patients. This result indicated that the NLR value could predict disease progression.

Previously, the relationship between increased NLR and mortality in various diseases has been reported.[25,29,30] In this study, we also saw significant differences between the survived and expired groups in the NLR (p<0.001). We found that the NLR is significantly higher in the expired group than in survived patients. Overall survival rate was lower in patients with higher NLR and dNLR ratios (NLR>10, dNLR<2.26) compared with those with the lowest ones (NLR<3.5 and dNLR>2.26).

A study in Turkey on 80 patients reported that NLR was significantly elevated in COVID-19 patients. They also showed that patients with NLR≥2.4 were 20 times more likely to be infected with the COVID-19 virus than patients below this ratio.[31] Furthermore, the NLR ratio related to inflammation and innate immune responses can help discriminate between severe and non-serious COVID-19 patients.[32]

There was a correlation between NLR and dNLR increase with other inflammatory mediators such as LDH, D-dimer, and ferritin. This study also found a correlation between the increased value of NLR and other inflammatory mediators such as LDH, D-dimer, and ferritin. According to the correlation coefficient, the correlation of NLR and dNLR values with LDH and D-Dimer (U/mL) was strong. Still, in the case of IL6, according to the correlation coefficient, the correlation was low. It should be noted that IL6 has been evaluated in Inpatients receiving anti-inflammatory and immunosuppressive drugs that can cause a decrease in IL6. More studies should be done in large numbers, including mild disease and low-risk patients.

LDH, D-dimer, ferritin, and CRP were associated with increased NLR and dNLR. There was a linear association between different NLR cut-offs and LDH, D-dimer, ferritin, and IL-6.

A correlation of increased NLR value with other inflammatory mediators such as LDH, ferritin, and D-dimer (U/mL) in our study was consistent with the previous studies.[33] Steroid therapy might affect the plasmatic levels of IL6 in contrast with NLR, which may constitute suitable alternative markers to evaluate severe forms of disease when patients are already undergoing steroid therapy.[34]

Previous studies have demonstrated that elevated levels of inflammatory markers were correlated with outcomes in COVID-19.[35,36] The dysregulation of immune responses in severe viral infections may be related to increased susceptibility to bacteria, which is another reason to explain the increase in neutrophils.[37]

Another study concluded that NLR (predicting severity) and IL-6 (the prognosis of COVID-19 patients) have better predictive power than other inflammatory markers.[33] Therefore, an increase in the NLR indicates an increase in other inflammatory markers.

However, checking NLR is easier and more cost-effective than measuring serum CRP levels and has a potential benefit as an inflammatory marker in patients with COVID-19.[25] Even though it is an easy-to-get laboratory parameter, the main problem is determining a higher sensitivity threshold.[13]

A study on 1065 patients found that higher admission D-dimer levels and D-dimer trends are associated with mortality and severity of the disease.[38] Shang et al,[39] compared CRP and NLR as predictors for the severity of COVID-19 disease, and they reported that NLR was a better predictor. It was found that ferritin had a high serum concentration in expired COVID-19 patients with severe infection.[40] CRP and ESR were significantly elevated at the early stage of COVID-19 infection, but CRP is more sensitive than ESR for predicting the severity of the disease.[41] It was reported that NLR, LDH, ferritin, D-dimers, fibrinogen, and CRP were associated with COVID-19 infection and disease severity.[42]

We also assessed the association of NLR and dNLR levels with other inflammatory factors. A significant difference was seen between LDH, CRP, ferritin, and D-dimer in different NLR and dNLR groups. Besides, a linear association was seen between LDH, ferritin, D-dimer, and IL6 and NLR levels. From this point of view, the NLR measurement reflects the level of other inflammatory factors such as LDH, ferritin, and D-Dimer.

Most patients with COVID-19 infection undergo a mild complication and may require short-term hospitalization. However, some patients experience a severe clinical course and may suffer long-term intensive care and significant mortality.[43,44] Early detection of patients prone to severe form alleviates intensive care and can reduce mortality.[45] Still, appropriate scoring systems for determining the prognosis and severity of this infection have remained problematic, so searching for a new indicator is one of the most researched topics.[7]

Therefore, clinicians must achieve a timely and accurate assessment of the disease at an early stage of COVID-19. According to studies, NLR is the right choice, but further studies are needed for final approval.

NLR is a reliable marker of systemic inflammation reflected by increased neutrophils and decreased lymphocytes.[46-49] The NLR value can be calculated quickly, is easy to determine using a complete blood count, and is inexpensive.[50] Some studies have reported that the NRL could be used as a diagnostic marker in ARDS and chronic obstructive pulmonary disease (COPD).[51] NLR correlates with the severity of the clinical course in ICU patients and is higher in acute cases than in stable patients. Therefore, it has been suggested as a prognostic indicator.[52] Besides, it was observed that the NLR decreased in patients with exacerbated COPD after recovery. Consequently, it can be used to predict the disease progression and remission of an inflammatory state.[51] Most serum inflammatory markers like leukocyte count, ESR, and CRP level are used in routine clinical practice, while NLR is a rapid, easy, and better predictor than these standard parameters. [46,53]

The association between the NLR and the severity of COVID-19 has recently been reported; we also demonstrated this correlation with our results. In the present study, we assessed the prognostic role of NLR, MLR, PLR, and dNLR in the severity of COVID-19. The increase in NLR and dNLR ratios were associated with the severity of the disease. The highest percentages of NLR and dNLR were seen in critical patients compared to severe and moderate groups. The correlation between NLR and dNLR and other laboratory biomarkers and its ability to predict the exacerbation and mortality of COVID-19 was seen. Thus, in areas where facilities are unavailable or expensive to measure inflammatory factors, examining NLR and dNLR due to their low cost and error can also reflect the level of other inflammatory reactions. According to our result, we proposed NLR 3-10, NLR>10, and dNLR<2.26, dNLR >2.26 cut-off points for predicting the severity of the disease. Of course, a more significant number of the study population is needed to determine a more exact cut-off point.

We believe a combination of the NLR and dNLR could be quickly calculated and predict the prognosis of COVID-19. This factor can help determine appropriate care and potential candidates to follow COVID-19 patients, especially those with a worse prognosis.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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