



QUANTITATIVE ANALYSIS OF BIOCHEMICAL AND HEMATOLOGICAL MARKERS IN COVID 19 PATIENTS AT DEDICATED COVID CARE HOSPITAL IN SOUTHERN RAJASTHAN

Pathology

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ABSTRACT

Introduction: - Corona virus disease (COVID-19) is an emerging disease that threatens the biosecurity of all countries and spreading rapidly worldwide. Early diagnosis of the disease is essential because of high infectivity and mortality rates of COVID-19.

Aims and objective: -The study was to observe and compare the clinical, biochemical and hematological profiles between high dependency unit (HDU), Intensive care unit (ICU) and pregnant women with confirmed COVID 19 positive RT-PCR.

Material and methods: - This study is conducted in COVID-19 dedicated Tertiary Care Hospital attached to RNT Medical College, Udaipur of Southern Rajasthan. Data of all patients with confirmed positive RT-PCR was collected during April 26, 2021 to May 31, 2021 from respective Lab Coordinator appointed for this purpose. SPSS statistics software was used for data statistics and mapping. Continuous variables were expressed as median and IQR (Inter Quartile Range).

Results:- Out of 428 patients 174 patients were in ICU, 235 patients were in HDU and 19 patients were pregnant women. Mean age of 428 is 47.32 year ranging 18-86yrs. Among these 428 patients 267(62.38%) patients were males and 161(37.62%) patients were females. The biochemical, coagulation and hematological parameters such as Serum Ferritin, Lactate Dehydrogenase, C-Reactive Protein, Blood Sugar, D-Dimer, Prothrombin Time & INR, ESR, WBC, and Absolute Neutrophil Count were statistically significant ($p < 0.05$) across all comparisons (ICU, HDU and pregnant women). IL6 (Interleukin-6) and Hemoglobin were significantly ($p < 0.05$) lower in pregnant women as compare to ICU patients. Absolute lymphocyte count, Platelet, Platelet lymphocyte ratio were significantly ($p < 0.05$) lower in HDU patients as compare to ICU patients.

Conclusion:- The present study has evaluated the clinical utility of biochemical parameter such as Ferritin, CRP, LDH, D-Dimer, and hematological markers like WBC, Neutrophils and Lymphocytes as significant predictors of COVID-19 outcome.

KEYWORDS

CBC, CRP, D-Dimer, Ferritin, IL6, Neutrophil Lymphocyte Ratio, Platelet lymphocyte ratio

INTRODUCTION

Coronavirus disease 2019 (COVID-19) was first detected in Wuhan, China, in December 2019 which is a highly infectious disease. In India first case was detected in Kerala on 30 January 2020 in a student who returned from Wuhan.⁽¹⁾

In March 2020, WHO (World Health Organization) declared Coronavirus disease 2019 (COVID-19) as a global pandemic.⁽²⁾

WHO has reported about mild to moderate symptoms in 80% of infected people, 13.8% have moderate infections and 6.2 % have severe infections.⁽³⁾

Mild to moderate case presented mostly as flu like complaints, fever, cough and a sore throat, followed less often by myalgia, malaise, fatigue, chest pain and headache.⁽⁴⁾

Severe illness is designated when patients have severe respiratory distress or pulse oximeter oxygen saturation $< 93\%$ on room air, respiratory rate > 30 breaths/min, respiratory infection and fever. Patients progressed rapidly to ARDS, metabolic acidosis, acute respiratory failure, coagulopathy and later on septic shock when Severely-ill.⁽⁵⁾

Patients admitted in Intensive Care Unit (ICU) showed leukocytosis with neutrophilia, lymphopenia and thrombocytopenia which were associated with greater fatality.⁽⁶⁾

This pandemic caused severe mortality and morbidity among the people as well as affected the economy of the world badly. The corona

virus is very infective and lethal hence to reduce its spread social distancing, masks and frequent hand washing are advised.

As there is rapid deterioration of patients of Covid 19, early diagnosis of patient is necessary. Blood tests provide information to physician about the inflammatory process and help them in early diagnosis.⁽⁶⁾

Complete blood counts (CBC) are cost-effective and easily available investigation. CBC parameters like white cell count, neutrophil count, lymphocyte count, platelet count and certain ratios (NLR, PLR, SII) can be used as inflammatory parameters. Biochemical markers such as IL6, Ferritin, CRP, LDH and D-dimer were representing inflammation and the immune system have been considered as a prognostic indicator in COVID-19-positive patients.

As we know the second wave of corona is very lethal and very small numbers of studies conducted specially on lab markers. Therefore, we planned study on the hematological and biochemical markers in COVID patients to help the clinicians for early diagnosis, treatment and follow up of patients.

The objective of the study was to observe and compare the clinical, biochemical and hematological profiles of patients admitted in High Dependency Unit (HDU), Intensive Care Unit (ICU) and Janana ward.

MATERIAL AND METHODS-

This study is conducted in COVID-19 dedicated Tertiary Care Hospital attached to RNT medical college, Udaipur of Southern Rajasthan. Data of all patients admitted during April 26, 2021 to May 31, 2021 from respective lab coordinator appointed for this purpose.

Thus, our study focuses on laboratory parameters of moderate to severe patients. The patient's data was analyzed based on their admission to Intensive Care Units (Severe), High Dependency Units (Moderate) or Janana ward (pregnant women).

INCLUSION CRITERIA:- All moderate to severe patients admitted to COVID wards and ICU with confirmed Covid 19 positive RT-PCR.

EXCLUSION CRITERIA:- OPD patients isolated at home and patients with incomplete documentation of laboratory testing were excluded from our study.

High Dependency Units (HDU):- All patients who were on Oxygen and on Bains circuit and maintaining saturation were included in HDU.

Intensive Care Units (ICU):- All patients (1)- who required mechanical Ventilation or on Bi-PAP(Bilevel Positive Airway Pressure) machine, (2)-patients in circulatory collapse who required cardiac monitoring (3) patients with severe Respiratory distress or Oxygen saturations <60%.

Data collection

The basic demographic details were collected from the admission records of our hospital. Whole blood samples were sent in EDTA vial for complete blood counts, plain vial for biochemical markers and citrate vial for coagulation parameters.

Neutrophil Lymphocyte Ratio (NLR) = Absolute Neutrophil Count (ANC)/Absolute Lymphocyte Count (ALC).

Platelet Lymphocyte Ratio (PLR) = Absolute Platelet (PLT) Count /Absolute Lymphocyte Count.

Systemic Inflammatory Index (SII) = Absolute Platelet Count (PLT) × Absolute Neutrophil Count/ Absolute Lymphocyte Count.

STATISTICAL ANALYSIS

SPSS statistics software was used for data statistics and mapping. Age was represented in mean (range), and gender in frequency and percentage. Continuous variables were expressed as median and IQR(Inter Quartile Range). For data with non-normal distribution, the Kruskal-Wallis test was used. The diagnostic values of the parameters for differentiating severe cases of COVID-19 patients were assessed by receiver operating characteristic (ROC) and area under the ROC curve (AUC). The 95% CI was calculated whenever appropriate, and a two-tailed $p < 0.05$ was considered statistically significant.

RESULTS

Total 947 patients were admitted in dedicated covid care hospital in

Udaipur of Southern Rajasthan during 26 April 2021 to 31 May 2021, out of which 428 patients were taken in our study for final statistical analysis. Out of 428 patients 174 patients were in ICU, 235 patients were in HDU and 19 patients were pregnant women.

Mean age of 428 is 47.32 year ranging (18-86 yrs). Mean age of ICU patient was 48.11 year ranging (18-83 yrs), mean age of HDU is 48.38 year ranging (19-86 yrs) and mean age of pregnant women was 26.95 year ranging (22-37 yrs).

Among these 428 patients 267 (62.38%) patients were males and 161 (37.62%) patients were females. (Table 1)

Table 1. Age & Sex details

SN	Age group	Male patients	Female patients	Total patients	% Percentage
1	0-20	1	1	2	0.47%
2	21-40	84	69	153	35.75%
3	41-60	125	63	188	43.92%
4	61-80	54	26	80	18.69%
5	81 and above	3	2	5	1.04%
6	Total patients	267 (62.38%)	161 (37.62%)	428	100%

The most common presenting symptom was shortness of breath followed by, continuous fever, sore throat, dry cough, loss of smell & taste sensations, rhinorrhea, chest pain, malaise/fatigability headache, and diarrhoea. (Table 2)

Table- 2. Symptoms at the time of admission

SN	Symptoms	Total patients n=428	% Percentage
1	Shortness of breath	287	67.05%
2	Continuous fever	238	55.60%
3	Sore throat	193	45.09%
4	Dry cough	147	34.35%
5	Loss of taste & smell sensations	89	20.79%
6	Rhinorrhea	43	10.04%
7	Chest pain	29	6.78%
8	Malaise/fatigability	23	4.77%
9	Headache	15	3.50%
10	Diarrhoea	7	1.63%

SN-Serial Number

Of the biochemical and coagulation parameters Serum Ferritin, Lactate Dehydrogenase, C-Reactive Protein, Blood Sugar, D-Dimer, Prothrombin Time and INR were statistically significant ($p < 0.05$) across all comparisons (ICU, HDU and pregnant women). (Table 3)

Table 3. Showing values of different Biochemical parameters among the patients of ICU, HDU Pregnant women

SN	Biochemical Marker with Normal range	Total patients Median (IQR) n=428	Pregnant women Median (IQR) n=19	HDU patients Median (IQR) n=235	ICU patients Median (IQR) n=174	P Value Total patients n=428	P Value HDU verses ICU n=409	P Value Pregnant verses ICU n=193
1	IL6 (0-4.4pg/ml)	30.625 (12.1-78.6)	16.74 (5.2-37)	26.7 (11.1-79.4)	34.785 (13.7-89.0)	0.142	0.119	0.037
2	S.Ferritin (13400ng/ml)	581.2 (274.6-1137.3)	205.6 (48.8-404.4)	473 (254.7-980.5)	769.2 (377.7-1435.8)	<0.001	<0.001	<0.001
3	LDH (81-234u/l)	635 (494.3-889.5)	465 (384-560.5)	601 (465.5-826)	753 (555.3-1033.0)	0.001	0.002	0.003
4	CRP (0-5mg/l)	66.65 (26.2-117.5)	27.6 (13.6-86.6)	58.3 (22.4-105.2)	82.45 (42.8-149.7)	0.001	<0.001	<0.001
5	B. Sugar (70-110Mg/dl)	114.5 (81-168.3)	91 (68-120.5)	107 (81-154)	125 (86.3-203.3)	0.003	<0.001	0.002
6	D- Dimer (0-0.5mg/l)	1.92 (0.82-3.95)	0.57 (0.51-0.96)	1.17 (0.6-2.33)	3.735 (2.03-10.37)	<0.001	<0.001	<0.001
7	P T in second	14.75 (13.5-16.5)	13.8 (13.5-14.4)	14.2 (13.1-15.7)	16.1 (14.2-18.5)	<0.001	<0.001	0.001
8	INR (<1.1)	1.11 (1.0-1.3)	1.02 (1.0-1.1)	1.07 (1.0-1.2)	1.2 (1.1-1.4)	<0.001	<0.001	0.005

SN-Serial Number, IL6 -Interleukin, S.Ferritin- Serum Ferritin, LDH- Lactate Dehydrogenase, CRP- C-reactive protein, B. Sugar- Blood Sugar, PT-Prothrombin time and INR -International Normalized Ratio.

In hematological parameters ESR, WBC, and Absolute Neutrophil Count, were statistically significant ($p < 0.05$) across all comparisons (ICU, HDU and pregnant women). (Table 4)

Table 4. Showing values of different Hematological parameters among the patients of ICU, HDU Pregnant women.

SN	Hematological Markers with Normal range	Total patients Median (IQR) N=428	Pregnant women Median (IQR) n=19	HDU patients Median (IQR) n=235	ICU patients Median (IQR) n=174	P Value Total patients n=428	P Value HDU verses ICU n=409	P Value Pregnant verses ICU n=193
1	ESR mm/hr	19 (16-21.3)	16 (15-17)	18 (16-20)	20 (18-22)	<0.001	<0.001	<0.001
2	Hb (11-16g/dl)	12.3 (11.0-13.6)	10.7 (8.7-11.3)	12.4 (11.1-13.7)	12.3 (11.2-13.7)	<0.001	0.659	<0.001
3	WBC (4-11) $\times 10^3/\mu\text{l}$	11.1 (7.7-15.3)	9.1 (6.6-17)	10.3 (7.3-13.7)	12.85 (9.6-19.2)	<0.001	<0.001	0.024
4	ANC (2-7.7) $\times 10^3/\mu\text{l}$	8.425 (5.7-11.5)	7.5 (4.5-12.2)	7.5 (5.3-10.2)	9.75 (7.1-14.5)	0.001	<0.001	0.036
5	ALC (0.6-4) $\times 10^3/\mu\text{l}$	1.5 (0.8-2.4)	1.4 (0.8-2.2)	1.4 (0.8-2.3)	1.7 (1.0-2.5)	0.244	0.022	0.292
6	% Neutrophil	75 (68-81.2)	72.5 (66.4-82.4)	75.3 (66.9-81.1)	75.05 (69.7-82.4)	0.774	0.154	0.623
7	% Lymphocyte	13.7 (9.8-19.0)	16 (9.6-21.1)	13.9 (10.1-20.5)	13.05 (9.7-17.7)	0.772	0.104	0.546
8	Platelet (150-400) $\times 10^3/\mu\text{l}$	176.5 (114.8-245.3)	175 (117.5-230.5)	188 (124-255)	163 (106-230.5)	0.049	0.017	0.832
9	NLR (0.78-3.53)	5.683333 (3.7-8.6)	5.5 (3.5-9.0)	5.6 (3.3-8.6)	5.77 (4.1-8.6)	0.833	0.205	0.683
10	PLR (36-172)	120.1089 (66.8-209.1)	151.8182 (65.7-210.8)	131.25 (73.9-224.6)	105.37 (50.6-168.2)	0.022	0.001	0.328
11	SII ($<390 \times 10^3/\mu\text{l}$)	970.1071 (550.3-1667.8)	958.3824 (493.6-1480.7)	1026.667 (572.3-1742.5)	919.55 (544.6-1628.7)	0.682	0.371	0.883

SN-Serial Number, ESR-Erythrocyte Sedimentation Rate, Hb-Hemoglobin, WBC-White Blood Cell, ANC-Absolute Neutrophil Count, ALC-Absolute Lymphocyte Count, NLR- Neutrophil Lymphocyte Ratio, PLR- Platelet Lymphocyte Ratio, SII- Systemic Inflammatory Index.

IL6 and Hemoglobin were significantly ($p < 0.05$) lower in pregnant women as compare to ICU patients.

Absolute lymphocyte count, Platelet count, Platelet lymphocyte ratio were significantly ($p < 0.05$) lower in HDU patients as compare to ICU patients.

Prothrombin time ($p < 0.001$), INR ($p < 0.001$) and D-dimer, ($p < 0.001$) were significantly higher in patients who were in ICU than the in patients those with HDU and Janana ward.

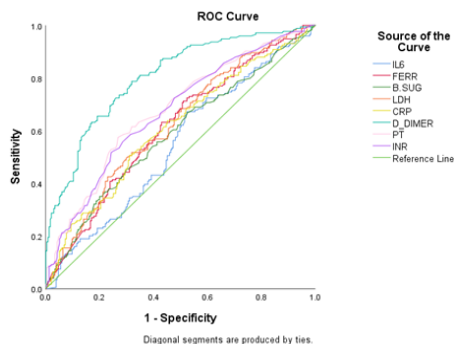


Fig-1 ROC Curve of Biochemical Marker in HDU versus ICU patient

Table -5. Of ROC Curve of Biochemical Marker HDU verses ICU patient

Test Result Variable (s)	Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
Il6	.545	.029	.119	.489	.601
FERR	.613	.028	.000	.558	.668
B.SUG	.588	.029	.002	.532	.644
LDH	.624	.028	.000	.570	.679
CRP	.612	.028	.000	.556	.667
D-DIMER	.805	.022	.000	.762	.847
PT	.678	.027	.000	.626	.731
INR	.672	.027	.000	.620	.725

IL6 - Interleukin6, FERR-Serum Ferritin, LDH- Lactate Dehydrogenase, CRP- C-reactive protein, B. Sugar- Blood Sugar, PT- Prothrombin time and INR - International Normalized Ratio.

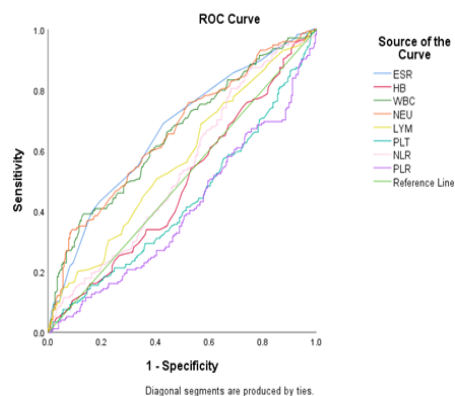


Fig-2 ROC Curve of Hematological Marker in HDU verses ICU patient

Table - 6. Of ROC Curve of Hematological Marker HDU verses ICU patient

Test Result Variable(s)	Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
ESR	.664	.027	.000	.611	.717
HB	.487	.029	.659	.431	.544
WBC	.653	.027	.000	.600	.707
NEU	.659	.027	.000	.606	.713
LYM	.566	.029	.022	.510	.622
PLT	.431	.029	.017	.375	.488
NLR	.537	.029	.205	.481	.593
PLR	.403	.028	.001	.347	.459

ESR-Erythrocyte Sedimentation Rate, Hb- Hemoglobin, WBC-White Blood Cell, NEU-Neutrophil Count, LYM-Lymphocyte Count, NLR-Neutrophil Lymphocyte Ratio, PLT- Platelet Lymphocyte Ratio, SII-Systemic Inflammatory Index.

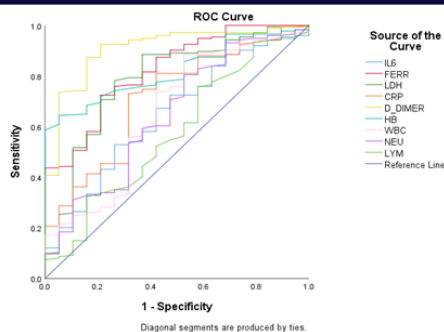


Fig.3 - ROC Curve of Biochemical & Hematological Marker in Pregnant women verses ICU patients.

Table – 7. Of ROC Curve of Biochemical & Hematological Marker in Pregnant women verses ICU patients.

Test Result Variable(s)	Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
Il6	.646	.065	.037	.520	.773
FERR	.823	.047	.000	.730	.915
LDH	.786	.059	.000	.669	.902
CRP	.712	.061	.002	.592	.832
D-DIMER	.907	.034	.000	.840	.974
HB	.819	.036	.000	.749	.889
WBC	.657	.071	.024	.518	.797
NEU	.647	.069	.036	.511	.783
LYM	.574	.071	.292	.434	.713

DISCUSSION

Corona virus disease (COVID-19) is an emerging disease that threatens the biosecurity of all countries and spreading rapidly worldwide.⁽⁵⁾ Early diagnosis of the disease is essential because the high infectivity and mortality rates of COVID-19.⁽⁶⁾ So it is useful to analyse biochemical and hematological markers at an early stage so that timely treatment can be started. They also play an important role to predict the severity and mortality of COVID-19 disease.

In our study large proportion of patients were HDU/ICU patients as this is a COVID-19 dedicated Tertiary Care Hospital and handles majority of moderate to severe cases of southern Rajasthan.

Younger ages are affected more severely than the older population. This may be due to the fact that older population had received vaccination which in contrast to in Nag A. et al study.⁽⁴⁾

Females are less affected than males probably because of males tend to go outside for different purposes more than female similar finding were noted in Liao D. et al.⁽⁷⁾

Our study has noted Shortness of breath, fever, sore throat and dry cough as the major symptoms prevalent among patients similar finding were noted in Krishnegwda et al study.⁽⁸⁾

In our study, high level of IL-6 with median value 30.625 similar finding were noted in the study of Yesupatham ST et al.⁽⁹⁾ The elevated IL-6, ferritin levels are probably due to severe cytokine release syndrome and secondary phagocytic lymphohistiocytosis which damage to other vital organs similar to Velavan TP et al study.^(8,9,10,11)

The increase in LDH levels observed in our study is consistent with the findings of Liu who correlated LDH, CRP, neutrophil and lymphocyte abnormalities with severe COVID pneumonia.⁽¹²⁾

The increase in CRP levels reflects the extent of the systemic inflammatory syndrome. It is accompanied by acute tissue damage with the onset of severe ARDS and subsequent multi-systemic failure in severe forms of the disease similar to Duarte FB et al study.⁽¹³⁾

Our study showed significant elevation of D-dimer, PT and INR in the HDU/ICU patients. An elevated D-dimer level was the cause of coagulopathy with thrombo-embolic complications which was significantly related to severity in our study population. COVID-19 cause increased D-dimer during disease progression. An outcome of

death was associated with prolonged prothrombin time, increased D-dimer, increased neutrophil lymphocyte ratio and thrombocytopenia.⁽⁷⁾ In this study, amongst pregnant women hemoglobin was low with a median value of 10.7gm/dl, as compare to HDU and ICU patient.

In our study, TLC was raised in ICU, HDU and pregnant women. Differential leukocyte count showed neutrophilia, which is indicated by median value ANC being 8.425 and lymphocyte count towards lower range, with median value ALC being 1.5. These findings are in concordance with study done by Bharat VN et al.⁽³⁾

Lymphopenia, which has also been documented in covid-19, seems to be the most relevant peripheral hematopoietic alteration, its use being suggested as a severity biomarker of the infection.⁽¹³⁾ Suggested causes were that interleukin (IL)-6 cytokines, tumor necrosis factor (TNF) α could induce lymphocyte deficiency and the virus could directly destroy lymphatic organs like thymus and spleen.⁽¹⁴⁾ Majority of patients had leukocytosis with neutrophilia so we attribute lymphopenia to be relative lymphopenia.

In this study Platelet count were towards lower side normal range, with a median value $176.5 \times 10^3/\mu\text{l}$. Platelet destruction by the immune system or due to synthesis inhibition can lead to thrombocytopenia, similar finding were noted in the study Kantri A et al.^(5,15)

Our results showed that high neutrophil to lymphocyte ratio, can be useful for predicting severity and mortality. Which might be a result of excessive inflammation and immune suppression in sepsis triggered by SARS-CoV-2 infection, similar finding in Nag A. et al study.⁽⁴⁾

In present study elevated NLR of >5 , systemic immune-inflammation index of >500 , CRP, LDH, and D-dimer were also observed in most of the patients similar to Krishnegwda R study.⁽⁸⁾ Our finding implies that elevated PLR levels determining the severity and mortality of COVID-19 patients, may be due to an overactive inflammatory response, similar to study of Kartal, O et al.⁽¹⁶⁾

The SII has been proposed as a prognostic indicator in the follow-up case of sepsis patients, which relies on thrombocytes, neutrophils and lymphocytes. The SII is an index defining the instability in the inflammatory response, and it's importance as a prognostic score proposed recently.⁽⁶⁾

In our study pregnant female were mild to moderately affected either because of younger age ranging (22-37yrs) or may be due to X-chromosome which may be providing protection to from viral infections. X-chromosome were responsible for rendering innate, adaptive immunity to female.⁽³⁾ In our study, the AUC (Area Under the Curve) of ICU versus HDU shows IL6, S.Ferritin, LDH, CRP, Blood Sugar, D-Dimer, Prothrombin Time, INR, ESR, WBC, Neutrophil, Lymphocyte and NLR were above the references line thus, they are effective and have good predictive value for predicting COVID-19.

Table -8. Comparison p value of HDU verses ICU patient in different study

Variables	Present Study P value	Singh P et al ⁽²⁾ P value	Kantri A et al ⁽⁵⁾ P value	Nag A et al ⁽⁴⁾ P value	Eugene B et al ⁽¹⁷⁾ P value
Il6	0.119	NA	NA	NA	NA
Ferritin	<0.001	0.24	<0.001	NA	NA
LDH	0.002	0.95	<0.001	NA	0.003
CRP	<0.001	0.203	<0.001	<0.001	NA
D-Dimer	<0.001	0.03	0.001	<0.001	NA
Heamoglobin	0.659	0.92	0.433	0.0076	0.07
WBC	<0.001	0.01	0.051	NA	0.36
ANC	<0.001	0.006	<0.001	NA	0.17
Lymphocyte	0.022	0.6	0.003	NA	<0.001
Pletlet	0.017	0.26	0.565	0.9	0.67
NLR	0.205	0.004	NA	<0.001	NA
PLR	0.001	0.89	NA	NA	NA

P value <0.05 is significant. NA-not available

Limitation

Our study has some limitations some patients were excluded because of incomplete documentation of laboratory testing. In addition, the study has not evaluated the outcomes as treatment details, intervention

and mortality.

CONCLUSION

The present study has evaluated the clinical utility of biochemical parameter such as Ferritin, CRP, LDH, D-Dimer, PT, INR and hematological marker like WBC, Neutrophils, Lymphocytes, Platelet, PLR as significant predictors of COVID-19 outcome.

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