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ORIGINAL RESEARCH

Comprehensive analysis of bio-markers in COVID-19 patients to predict the duration of hospitalization

[1]Shrishti Dhar Prasad, [2]Suprava Patel,[3]Ajoy Kumar Behera,[4]Rachita Nanda,[5]Eli Mohapatra

[1]Department of Biochemistry, Uttar Pradesh university of Medical Sciences, Saifai, Etawah, Uttar Pradesh, India
[2,4,5]Department of Biochemistry, All India Institute of Medical Sciences, Raipur, Chhattisgarh, India
[3]Department of Pulmonary Medicine, All India Institute of Medical Sciences, Raipur, Chhattisgarh, India

Corresponding Author
Shrishti Dhar Prasad
Assistant Professor, Department of Biochemistry, Uttar Pradesh university of Medical Sciences, Saifai, Etawah, Uttar Pradesh, India

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ABSTRACT
Background: Strict monitoring of the changes in biomarkers might aid clinicians to monitor the clinical course of the SARS-CoV-2 infection and intervene accordingly. The study was aimed to evaluate the laboratory parameters in severe and non-severe COVID-19 cases and its utility in predicting the duration of hospitalization.
Methods: It was a retrospective study on seventy-nine COVID-19 patients. Data was collected for demographic details, clinical features and laboratory values for study analysis.
Results: The mean (SD) of duration of hospital stay was 9.7 (4.5) days for severe patients and 8.3 (4.2) days for non-severe cases (p=0.08). The odds for death was 12.38 times (CI95%:1.36-112.41) in severe COVID-19 patients than the non-severe ones. Serum biomarkers like urea, liver enzymes were significantly higher in severe group whereas total protein, albumin, lymphocytes and eosinophils were significantly lower. The mean CRP-Albumin ratio (CAR) value was found to be 2.5 times higher in them (p<0.001). Duration of hospitalization was increased with rise in serum urea (by 47%), CAR (51%), eosinopenia (by 12%) and lymphopenia (by 12%).
Conclusion: The findings suggested that close monitoring of these biomarkers can aid in improving recovery in patients of COVID-19.
Key words:SARS-CoV-2, serum markers, hemogram, immunoglobulins, complement factors, CAR, lymphopenia, eosinopenia

INTRODUCTION

The novel coronavirus disease (COVID-19) was declared as a pandemic owing to its world-wide rapid spread and posing a serious threat to the health of the people. Although, majority of the cases are of mild to moderate grade but few of them rapidly progress to severe grade and develop clinical manifestations of severe pneumonia, septic shock or acute respiratory distress syndrome (ARDS) resulting in mortality rate of 4.35-15% in such patients [1,2]. Any delay in diagnosis of the development of disease severity would delay the recovery rate and lengthen the hospital stay. Early intervention before the development of critical stage is highly crucial in order to reduce mortality [3]. Strict monitoring of the changes in biomarkers might aid clinicians to monitor the clinical course of the disease and actively intervene accordingly.

Several biomarkers such as C-reactive protein (CRP), lactate dehydrogenase (LDH), ferritin, interleukin-6 (IL-6), neutrophil-lymphocyte count, eosinophilia have been linked with the inflammatory condition of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, yet, there is no conclusive evidence of direct link between any specific biomarker and clinical severity [4-6]. It is suggested that combination of indicators might be more effective in predicting the clinical outcome than a single specific marker. More so, primary health centers, owing to lack of infrastructure cannot perform high end laboratory testing like ferritin, IL-6, D-dimer required for severity assessment of the disease. Hence, a

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comprehensive evaluation of the routine laboratory biomarkers is required that might help to assess the critical patients for appropriate therapeutic intervention and improve the recovery rate.

The study was aimed to evaluate the laboratory parameters in severe and non-severe cases to be used for prediction of recovery and thus the duration of hospitalization in admitted patients diagnosed with COVID-19.

MATERIALS AND METHODS

STUDY DESIGN AND POPULATION

The retrospective study included adult (>18 years) patients diagnosed with COVID-19 and hospitalized in the hospital during the one month of study period in September 2020. The cases were diagnosed as severe and non-severe on the basis of World Health Organization's (WHO's) interim guidelines [7]. The non-severe group included asymptomatic and/or symptomatic patients with oxygen saturation (SpO_2) more than equal to 90% on room air by pulse oximetry. Patients with SpO_2 less than 90% on room air were categorized under severe group. Of all the admitted cases, seventy-nine cases with clinical and all laboratory details were included for the study. Antenatal cases, lactating females, children, those with known case of autoimmune disorders, cancers, undergoing therapy for cancer, under immunosuppression therapy of any cause, blood transfused within last three months, blood sample analyzed after twenty-four hours of admission and those with incomplete medical records were excluded.

The institute ethics committee reviewed and approved the study with waiver of consent as patients' names remain coded. Guidelines for good clinical practice as per declaration of Helsinki were followed for the study.

CLINICAL AND LABORATORY DATA COLLECTION

The demographic details, clinical manifestations for signs and symptoms, presence of comorbidities, duration of hospital stay (HS), laboratory analyses and mortality details were reviewed from patient's case record in medical record section of the institute. Blood samples collected and reported within first twenty-four hours of admission were entered for analysis.

Each patient was scored according to the number of signs and symptoms, presence of number of comorbid conditions and mortality status. Adding up the numbers, a clinical score (CS) was assigned to each of them.

Serum routine biochemical markers such as urea (Ur), creatinine (Cr), uric acid (UA), sodium (Na^+), potassium (K^+), chloride (Cl^-), total bilirubin (TBil), direct bilirubin (DBil), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), total protein (TP), albumin (Alb), magnesium (Mg), phosphorous (Phos), calcium (Ca), C-reactive protein (CRP) and lactate dehydrogenase (LDH) were analyzed in fully automated clinical chemistry AU680 Beckman Coulter Inc. Serum ferritin (Fer) was analyzed in Advia Centaur XP from Siemens Healthineers. Serum immunoglobulins G (IgG) & M (IgM) and complement factors, C3 & C4 were processed in Mispa i3 from Agappe diagnostics. Complete blood count (CBC) was performed in XP-100 Fully Automated HematologyAnalyzer, Sysmex. Serum CRP, LDH and ferritin were considered as inflammatory markers in this article. All short forms have been enlisted in abbreviations table.

STATISTICAL ANALYSIS

Ratios were calculated for AST-to-ALT (AST/ALT), albumin-to-globulin (A/G), CRP-to-albumin ratio (CAR), neutrophil-to-lymphocyte ratio (NLR) and lymphocyte-to-monocyte ratio (LMR).

Categorical values were presented as frequency percentage (%) and compared between the groups using Chi-square test or Fishers' Exact test as applicable. All quantitative variables were expressed as mean with standard deviation (SD) irrespective of whether normally distributed or non-normally distributed in order to avoid confusions for the readers. However, the continuous variables were compared using independent samples *t*-test (for normally distributed variables) and Mann-Whitney *U* test (for non-normally distributed data). The correlation coefficients with their significance were evaluated by Spearman test. Poisson regression analysis was performed for all the laboratory and clinical factors to determine the relationship between the variables with the number of days of HS that was considered as the outcome variable as an indicator of recovery period. Using the receiver operating characteristics (ROC), area under curve (AUC) and cut-off values for number of days of HS of more than eight days were figured out. The statistical analysis was performed in SPSS software version 20 (IBM Corp.). Statistical value of p less than 0.05 was considered significant.

RESULT

FREQUENCY PERCENTAGES OF CATEGORICAL VARIABLES IN THE STUDY POPULATION

The retrospective observational analysis included fifty-three non-severe cases and twenty-six severe cases of COVID-19. The number of cases above and below fifty-five years of age did not differ significantly (p=0.91). Similarly gender distribution (p=0.19) and presence of comorbidities (p=0.63) between the two groups were not significantly different. However, total number of males (n=20) admitted under severe group were more than three times to that of females (n=6) (Figure 1). Diabetes mellitus (DM) was the most common comorbidity associated in the admitted cases (40.5%) followed by hypertension (30.4%). Breathlessness was the most

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common presentation in severe cases (92.2%) whereas cough was the commonest presenting symptoms in non-severe cases (52.8%). Number of deaths was significantly more in severe cases when compared to that of non-severe form (p=0.013) (Figure 1). The odds for death was 12.38 times (CI95%:1.36-112.41) in severe COVID-19 patients than the non-severe ones.

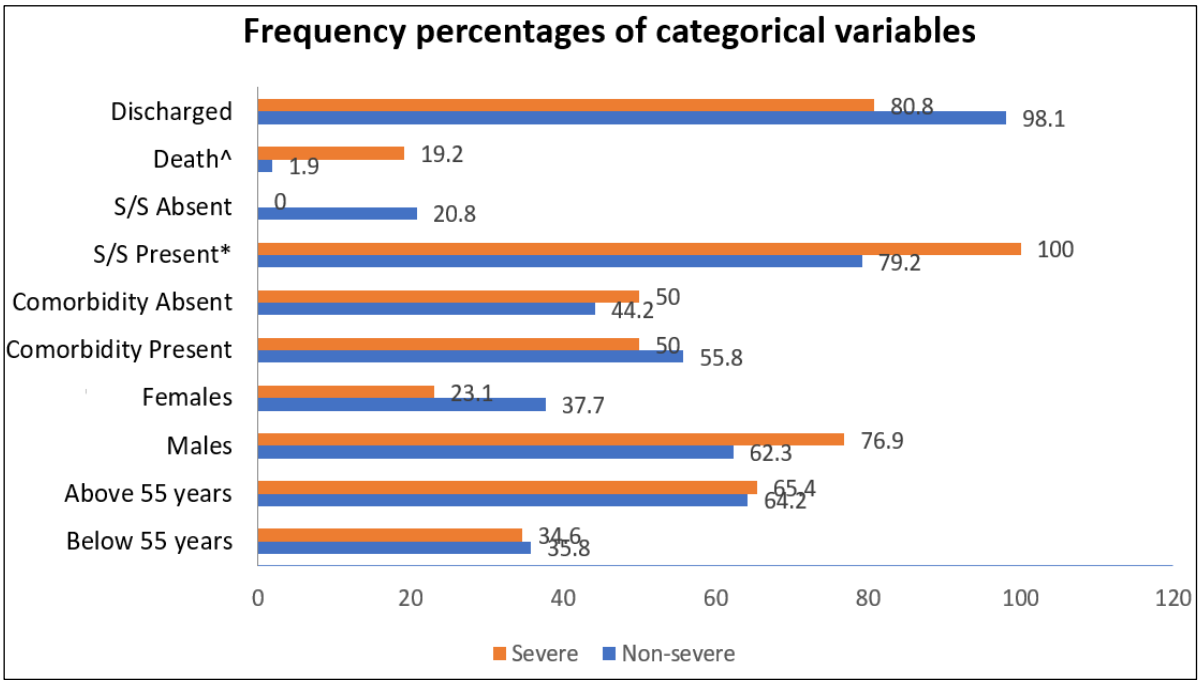

Figure 1: Frequency percentages of categorical variables in the study population

S/S: signs and symptoms during admission; * denotes significance at p=0.002 for Chi-square value for presence of signs and symptoms when compared to its absence in both the study groups; ^ denotes significance at p=0.006 for Chi-square value for number of deaths when compared to those discharged after treatment in both the study groups

MEAN (SD) VALUES OF NUMBER OF DAYS OF HOSPITAL STAY AND CLINICAL SCORE IN THE STUDY GROUPS

The mean (SD) of SpO_2 in non-severe and severe cases were 95.62% (2.4) and 77.46% (11.8) respectively. As reflected in Figure 2, the mean (SD) CS was 5.1 (1.6) for severe cases which was significantly higher than non-severe cases (p<0.001). The mean (SD) of duration of hospital stay was 9.7 (4.5) days for severe patients and 8.3 (4.2) days for non-severe cases (p=0.08).

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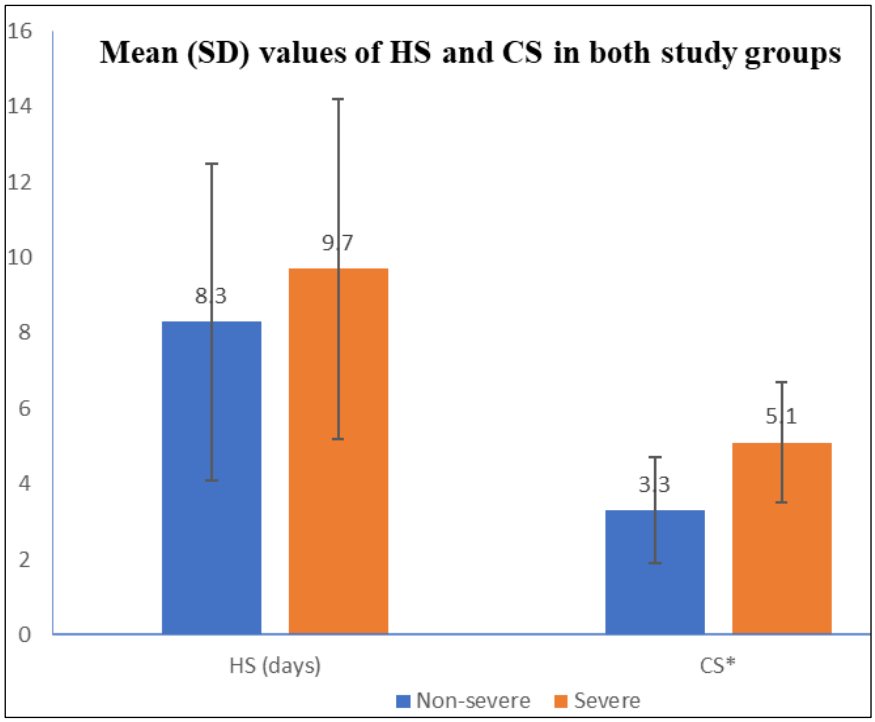

Figure 2: Mean (SD) values of number of days of hospital stay and clinical score in the study population

HS: number of days of hospital stay; CS: clinical score; *denotes significance at p<0.001 for CS compared between the two study groups

COMPARISON OF THE MEAN (SD) VALUES OF BIOMARKERS IN THE STUDY GROUPS

Table 1 illustrates the difference between the mean (SD) values of all the laboratory variables. Serum biomarkers like Ur, TBil, DBil, AST, ALT, ALP, GGT and Phos were significantly higher in severe group whereas TP (p=0.002) and Alb (p<0.001) were significantly lower. The serum inflammatory markers, CRP, LDH and Fer were significantly elevated in severe patients (p<0.001). The mean CAR value was found to be 2.5 times higher in them (p<0.001). Significant rise was also recorded for red cell distribution width (RDW), total leucocyte count (TLC), neutrophil count (NC), NLR, platelet count (PC) and erythrocyte sedimentation rate (ESR) whereas lymphocyte count (LC), LMR and eosinophil counts (EC) were reduced in severe cases.

The rise in mean NLR value was nearly 4.2 times in severe COVID-19 patients whereas the mean LMR was almost half when compared to the non-severe patients (p<0.001).

Table 1: Comparison of the mean (SD) values of biomarkers in the study groups

Lab variables (Unit)	Non-severe (N=53)	Severe (N=26)	P-value	Lab variables (Unit)	Non-severe (N=53)	Severe (N=26)	P-value
	Mean (SD)	Mean (SD)		IgM (mg/dL)	122.5(67.5)	120.6(86.9)	0.6
Ur (mg/dL)	31.5(17.2)	58.9(37.4)	<0.001*	IgG/IgM	21.17(26.5)	17.36(14.1)	0.72
Cr (mg/dL)	1.2(0.4)	1.4(0.9)	0.34	C3 (mg/dL)	176.4(59.7)	170.2(55.7)	0.43
UA (mg/dL)	5.2(2.5)	4.4(2.1)	0.136	C4 (mg/dL)	41.8(18)	38(20.9)	0.44
Na^+ (mmol/L)	139.1(5.3)	138.1(4.3)	0.39	C3/C4	4.67(2.04)	5.79(3.2)	0.062
K^+(mmol/L)	4.2(0.4)	4.4(0.7)	0.064	Hb (Gm/dL)	12.2(1.5)	12.2(1.8)	0.94
Cl^-(mmol/L)	104.6(4)	102.85(4.4)	0.073	HCT(%)	37.4(4.4)	37.1(5.3)	0.78
TBil (mg/dL)	0.74(0.5)	1.07(1.1)	0.013*	RBC ($x10^6$/L)	4.4(0.6)	4.3(0.9)	0.7
DBil (mg/dL)	0.21(0.2)	0.39(0.6)	0.002*	MCV (fL)	85.5(8.9)	86.7(9.4)	0.57
AST (U/L)	45.1(31.6)	77.5(96.5)	0.008*	MCH (pg)	27.9(3.5)	28.5(3.9)	0.49
ALT (U/L)	40.3(34.3)	76(98.8)	0.023*	MCHC (Gm/dL)	32.6(1.4)	32.8(1.7)	0.56
ALP (U/L)	60.8(55.5)	103.6(62.9)	<0.001*	RDW (%)	13.9(1.5)	15.2(3.7)	0.025*
AST/ALT	1.44(1.04)	1.35(0.82)	0.75	TLC (x 10^3/L)	7.9(3.5)	12.9(8.1)	<0.001*
GGT (U/L)	68.3(38.7)	116.2(66.5)	<0.001*	NC (%)	63.3(13.1)	79.7(11.6)	<0.001*
TP (Gm/dL)	7.12(0.7)	6.56(0.7)	0.002*	LC (%)	25.5(12.2)	11.8(8.3)	<0.001*
Alb (Gm/dL)	3.8(0.5)	3.1(0.4)	<0.001*	NLR	3.9(3.9)	16.15(18.1)	<0.001*

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A/G ratio	1.2(0.2)	0.93(0.2)	<0.001*	MC(%)	8.1(4.2)	6.7(3.8)	0.16
Mg (mg/dL)	2.2(0.3)	2.2(0.6)	0.93	LMR	3.81(2.5)	1.86(1.3)	<0.001*
Phos (mg/dL)	3.8(1.2)	5.1(2.5)	0.004*	EC (%)	2.95(1.9)	1.4(1.9)	0.001*
Ca (mg/dL)	8.8(0.6)	8.7(0.8)	0.43	BC (%)	0.006(0.04)	0.019(0.05)	0.23
CRP (mg/L)	46.3(58)	104.4(73.8)	<0.001*	PC (x 10^3/L)	232.4(108.7)	292.5(131.6)	0.055
LDH (U/L)	550.3(218.8)	889.5(383.8)	<0.001*	ESR (mm)	72.6(50.5)	101.7(54.1)	0.04*
Fer (ng/ml)	425.9(456.7)	823.4(531.1)	<0.001*	PT (sec)	10.7(1.2)	11.1(1.2)	0.15
CAR	1.3(1.7)	3.4(2.5)	<0.001*	INR	0.98(0.1)	1.02(0.1)	0.19
IgG (mg/dL)	1531.9(479.6)	1344.5(490.3)	0.21				

*Denotes significance at $p<0.05$; all full forms are enlisted under abbreviation section

POISSON REGRESSION ANALYSIS BETWEEN THE LABORATORY VARIABLES AND THE OUTCOMES VARIABLES

Poisson regression analysis (Table 2) depicted higher CS would increase the HS by 7% (95%CI:0.99-1.14). An increase in percentage of days of HS was seen to be associated with rise in serum Ur (by 47%), Cr (by 8%), Na^+ (by 2.1%), K^+ (by 22%), ALT (by 9.8%), AST/ALT (by 1.3%), GGT (by 95%), CRP (by 1.2%), CAR (51%), C4 (by 1.4%) and C3/C4 (by 2.8%). Unlike the above parameters, serum Alb and Mg depicted a protective effect of 57% and 26% respectively.

TLC (r=0.265; p=0.018), NC (r=0.248; p=0.028) and NLR (r=0.277; p=0.013) depicted significant positive correlation with that of HS and the duration was found to be increased by factor 1.015, 1.127 and 1.17 respectively.In contrary, LC (r= -0.139; p=0.22) and EC (r= -263; p=0.019) recorded negative correlation with HS. Lowered LC and EC affected the HS by 12%. PC and PT in turn showed protective effect towards HS as against INR values that increased the duration by a factor of 22.45.

Table 2: Poisson regression analysis between the laboratory variables and the outcomes variables

Variables	p-value	Exp(B)	95%CI	Variables	p-value	Exp(B)	95%CI
Age	0.46	0.995	0.98-1.009	CAR	0.096	1.51	0.93-2.44
Gender	0.039*	0.995	0.99-1.0	IgG (mg/dL)	0.38	1	
SpO2	0.21	1.011	0.99-1.028	IgM (mg/dL)	0.23	0.99	0.99-1.001
CS	0.05	1.07	0.99-1.14	IgG/IgM	0.51	0.99	0.99-1.004
Ur (mg/dL)	0.37	1.47	0.63-3.43	C3 (mg/dL)	0.19	0.99	0.99-1.001
Cr (mg/dL)	0.54	1.08	0.84-1.38	C4 (mg/dL)	0.002*	1.014	1.005-1.023
UA (mg/dL)	0.53	0.98	0.92-1.043	C3/C4	0.51	1.028	0.95-1.117
Na^+ (mmol/L)	0.221	1.021	0.99-1.055	Hb (Gm/dL)	0.85	1.19	0.21-6.9
K^+(mmol/L)	0.07	1.22	0.984-1.51	HCT(%)	0.61	0.836	0.42-1.68
Cl^-(mmol/L)	0.3	0.98	0.94-1.02	RBC (x10^6/L)	0.45	2.36	0.25-21.9
TBil (mg/dL)	0.029*	0.97	0.93-0.99	MCV (fL)	0.55	1.126	0.76-1.66
DBil (mg/dL)	0.78	0.84	0.24-2.94	MCH (pg)	0.68	0.822	0.33-2.054
AST (U/L)	0.87	1	0.995-1.005	MCHC (Gm/dL)	0.793	1.127	0.46-2.76
ALT (U/L)	0.48	1.098	0.85-1.42	RDW (%)	0.93	1.003	0.94-1.072
ALP (U/L)	0.91	1.013	0.78-1.29	TLC (x 10^3/L)	0.15	1.015	0.99-1.037
AST/ALT	0.76	1	0.997-1.002	NC (%)	0.19	1.127	0.944-1.347
GGT (U/L)	0.595	1.94	0.17-22.19	LC (%)	0.21	1.121	0.938-1.34
TP (Gm/dL)	0.188	0.995	0.99-1.002	NLR	0.105	1.17	0.97-1.41
Alb (Gm/dL)	0.27	0.43	0.094-1.95	MC(%)	0.45	1.072	0.895-1.283
A/G ratio	0.091	0.95	0.89-1.009	LMR	0.93	0.994	0.86-1.16
Mg (mg/dL)	0.011*	0.74	0.59-0.94	EC (%)	0.19	1.126	0.941-1.35
Phos (mg/dL)	0.032*	1.002	1-1.004	BC (%)	0.64	0.515	0.032-8.31
Ca (mg/dL)	0.29	0.92	0.79-1.07	PC (x 10^3/L)	0.001*	0.996	0.994-0.998
CRP (mg/L)	0.018*	1.012	1.002-1.021	ESR (mm)	0.017*	1.001	1.0-1.002
LDH (U/L)	0.99	1	---	PT (sec)	0.006*	0.63	0.45-0.88
Fer (ng/ml)	0.4	1	---	INR	0.04*	22.45	1.159-434.8

*Denotes significance at $p<0.05$; all full forms are enlisted under abbreviation section

ROC CURVE, AUC AND CUT-OFF VALUES OF INDEPENDENT RISK FACTORS FOR DURATION OF HS

ROC curve and cut-off values were determined for all the variables to find out the independent predictors for increase in HS of more than eight days. ROC curve that reflected an AUC of more than 0.65 are depicted in Figure 3 and the AUC with cut-off values for those parameters are depicted in Table 3. Sensitivity of serum urea was 85.7% at 35.5mg/dL and specificity

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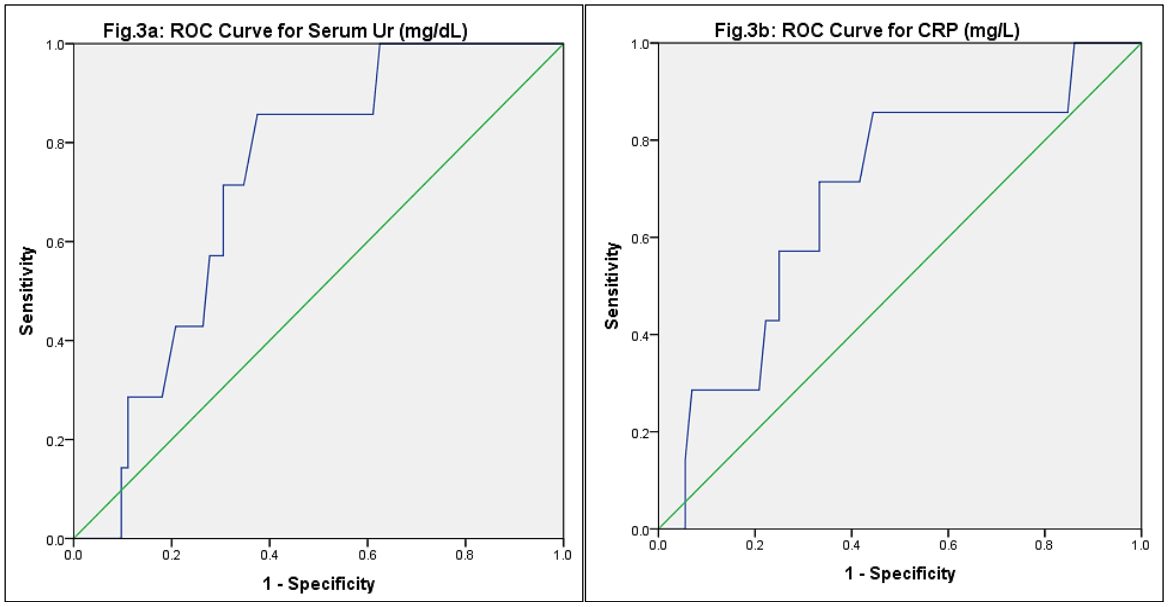
was 62.5%. 71.4% and 66.7% was the sensitivity and specificity respectively for CRP at 60 mg/L. The sensitivity and specificity was around 71% for CAR at a value of 1.7. Lymphopenia tend to increase the duration of stay and depicted a sensitivity of more than 85.7% and specificity of 62.5% at a value of 17.1%.Similarly, low EC count significantly increased the duration of HS with an AUC of 0.739 (p=0.038). The respective sensitivity and specificity for EC was 71.4% and 62.5% at a value of 1.6%. ESR of 67 depicted 100% sensitivity whereas for a value of 98, the sensitivity was 71.4% and specificity was 63.1%.

Table 3: AUC and cut-off values of independent risk factors for HS

Parameter	AUC	SE	P-value	Cut-off Value	Sensitivity	Specificity
Ur (mg/dL)	0.72	0.075	0.056	35.5	85.7%	62.5%
				45.5	57.1%	79.2%
CRP (mg/L)	0.686	0.102	0.11	41.5	85.7%	55.6%
				60.0	71.4%	66.7%
CAR	0.692	0.1	0.094	1.5	85.7%	61.1%
				1.7	71.4%	71.8%
LC (%)	0.66	0.9	0.17	17.1	85.7%	62.5%
				13.5	71.4%	69.4%
EC (%)	0.739	0.12	0.038*	1.6	71.4%	62.5%
				2.45	85.7%	52.8%
ESR (mm)	0.726	0.073	0.05*	67	100%	52.3%
				73	85.7%	55.4%
				98	71.4%	63.1%

AUC: Area under curve; SE: Standard error; *denotes significance at $p<0.05$; Ur: serum urea in mg/dL; CRP: C-reactive protein in mg/L; CAR: CRP-to-albumin ratio; LC: lymphocyte count in percentage; EC: eosinophil count in percentage; ESR: erythrocyte sedimentation rate in mm in one hour



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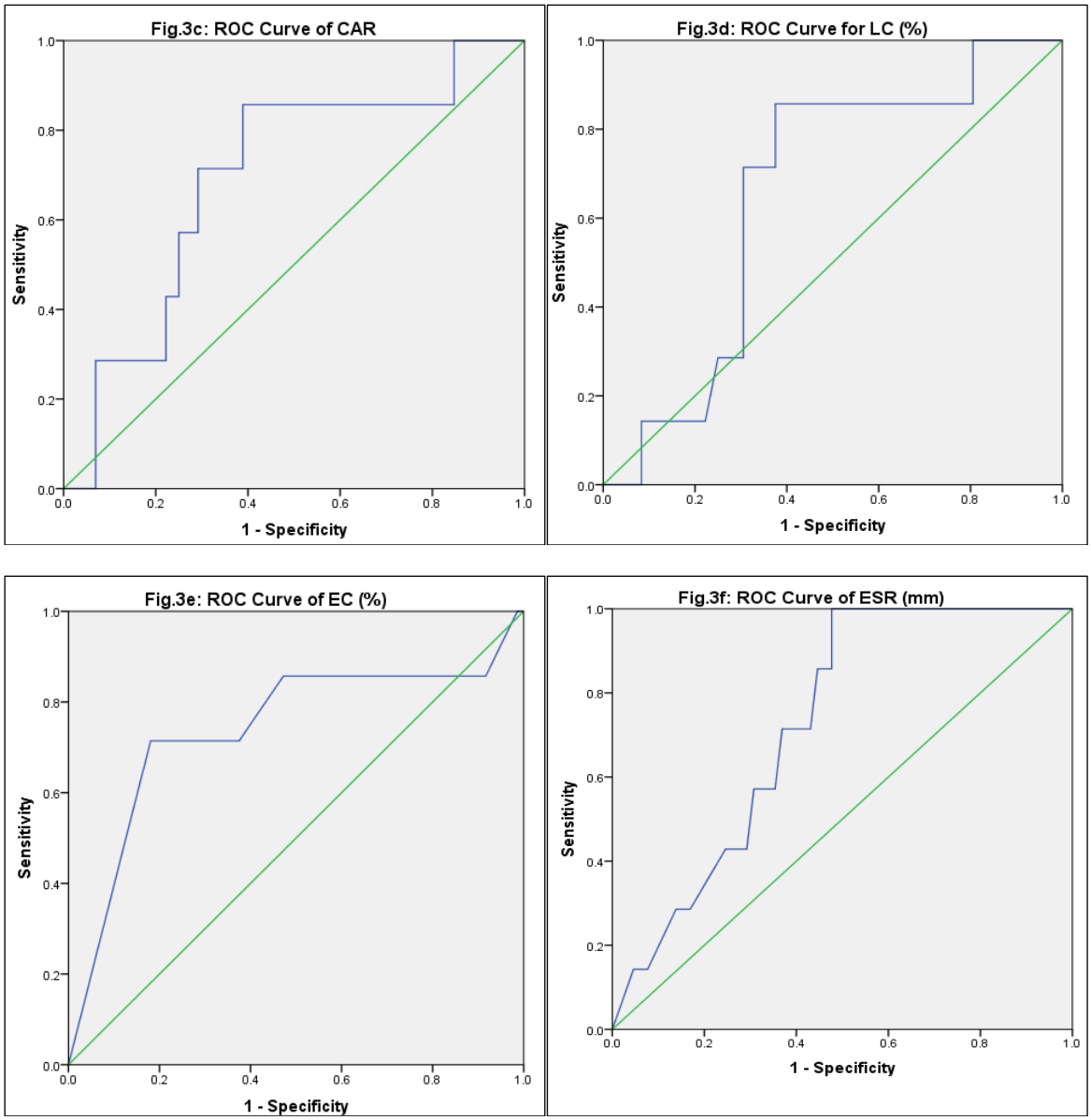

Figure 3: ROC curve of independent risk factors for duration of HS

ROC: Receiver operating characteristic; HS: Hospital stay; Ur: serum urea in mg/dL; CRP: C-reactive protein in mg/L; CAR: CRP-to-albumin ratio; LC: lymphocyte count in percentage; EC: eosinophil count in percentage; ESR: erythrocyte sedimentation rate in mm in one hour.

DISCUSSION

Studies have documented better prognosis for non-severe cases of COVID-19 and high mortality for severe cases. Hence, it is important to identify the rapid progression from non-severe form of the disease to severe form at an early stage in order to reduce mortality and improve recovery percentages in them.

The present retrospective observational analysis revealed that monitoring of biomarkers can warn regarding the severity of the disease and aid in timely intervention. This in turn would greatly reduce the recovery period and the duration of hospitalization of COVID-19 patients. Patients hospitalized in remote areas need to be monitored very closely as the infrastructure are not sufficient enough to provide them support during critical stage. High end instruments are usually not available for testing of specialized parameters like interleukin-6 (IL-6), D-dimer and immune markers nor do they have radiological diagnostic equipment to assess the severity. Hence, close monitoring of routinely estimated parameters might aid clinicians in risk stratification and monitoring the course of the disease in a COVID-19 patients in absence of sophisticated laboratory facilities.

The three serum inflammatory markers, CRP, LDH and ferritin, were increased in severe form of the disease and correlated positively with SpO_2 and CS values at the time of admission ($p<0.001$). However, the Poisson analysis depicted a significant impact of

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CRP towards increased HS. Higher CRP values tend to increase the number of days of hospitalization by 1.2%. The mean CRP value of in Zheng *et al*. study was 49.6mg/L in 32 critical patients whereas Asghar *et al*. study documented a mean of 198.67 mg/L in 101 non-survivors[8,9]. The mean value of the patients admitted in severe condition in the present study was 104.4 mg/L. The cut-off for CRP was 108.3 mg/L with 75.5% sensitivity and 56.7% specificity in Asghar *et al*. study. The present study depicted 85.7% sensitivity and 55.6% specificity at a CRP value of 41.5 mg/L. Unlike CRP, low serum albumin level is known as a poor prognostic marker for various inflammatory disorders. The inflammatory mediators like IL-6 and tumor necrosis factor-α (TNF-α) which in turn downregulates intrahepatic albumin synthesis or activates its catabolism [10,11]. Combination of two parameters definitely provides a better prognostic indicator as compared to individual analyte. Hence, the CAR index was taken into consideration to understand it's prognostic implication in recovery rate. Ranzani*et al*. study documented model-CAR that predicted mortality in intensive care unit. The sensitivity and specificity for mortality was more than two times when compared to CRP alone [12]. The mean CAR value was 1.66 for 84 severe cases in a study by Karakyoun*et al*. and the cut-off value of CAR was 0.9 with 69.1% sensitivity and 70.8% specificity ($p<0.001$) [11]. The present study recorded a mean value of 3.4 in severe cases and the cut-off value was 1.7 with a sensitivity and specificity of about 71% for more than eight days of HS. A large sample size data can produce a good AUC that can clearly make out CAR as a significant marker in early detection of COVID-19.

Besides inflammatory markers, few commonly investigated analytes like urea, AST, ALT and GGT documented significant differences between sever and non-severe forms of the disease. These parameters also depicted significant negative correlation with SpO_2 levels during admission ($p<0.001$) (data not shown). In contrary, TP, Alb and A/G ratio were found significantly reduced in severe COVID-19 cases and correlated positively with initial SpO_2 values ($p<0.01$).The explanation resides in the fact that the SARS-CoV-2 virus might be responsible for organ specific inflammation [13]. The Poisson analysis depicted that an increase in urea would tend to increase the HS by 47%. The mean (SD) urea level was 70.15 (51.83) mg/dL for non-survivor patients in Asghar *et al*. study [9]. The blood urea nitrogen (BUN) recorded was 4.02 mmol/L (51.7 mg/dL on conversion) in Wang *et al*. study [4]. The mean (SD) of urea in present study was 58.9 mg/dl that is quite similar to that of Wang *et al*. study. Urea value of 45.5mg/dL is nearly 80% specificity for increase in number of days of hospitalization as revealed by ROC curve in the present study.

Present study recorded a parallel increase of TLC, NC, NLR and ESR along with inflammatory biomarkers in severe cases whereas LC, LMR and EC depicted significant reduction. The findings were in agreement to Liu *et al*. study that recorded significantly higher NC and NLR and lower LC in 13 severe COVID-19 diseases when compared to 27 mild cases [14].Various studies have documented significant association of these markers with severity of the disease and NLR was identified as powerful predictor for prognosis of the disease [14-17]. Rise in NC and NLR depicted in the present study showed higher probability towards delayed recovery by 12% and 17% respectively. Similarly, lower LC and EC would tend to increase HS by about 12%. Unlike neutropenia, lymphopenia was found to be more prevalent in severe cases than the non-severe cases (76.9% vs 39.6%) in the present study. Recent published data reported that SARS-Cov-2 virions might affect the T-lymphocyte subsets resulting in the pathological changes due to the infection (14,18,19). The higher rate of lymphopenia seen in the severe cases might be due to significant fall in T cells which needs to be further elucidated in large scale longitudinal studies. The study also confirmed higher prevalence of eosinopenia (57.7% vs 15.1%) which accorded to the findings of Zhang *et al*. study that reported lymphopenia and eosinopenia in 75.4% and 52.9% cases [20].

Till now there are no standardized criteria for biomarkers for assessment of clinical severity in the COVID-19 patients. Thus, close monitoring of the changes in the laboratory markers might be the sole indicator of the clinical course towards severity so as to initiate appropriate therapeutic intervention and improve the recovery rate.

STUDY LIMITATIONS

Small sample size and the retrospective study design were the major limitations of the study. Although, some inflammatory biomarkers, immune indexes and coagulation profiles were not taken into consideration due to limitation of testing facilities, yet the major routine biomarkers have been analyzed which are routinely estimated in all peripheral health centers without any high-end equipment. Although, patients associated with immune related disorders or inflammatory disorders were excluded during case record selection, however, there might have been some selection bias in certain group of patients with secondary bacterial infection and that might not have been solely due to viral infection. Therefore, it is suggested that the readers must interpret the results with caution.

CONCLUSION AND FUTURE PERSPECTIVE

The retrospective study was designed to study the effect of various biomarkers on the duration of hospitalization. The findings suggest that besides inflammatory markers, the liver enzyme biomarkers, CRP along with CAR, progressive lymphopenia and eosinopenia showed significant contribution towards HS and need to be closely monitored in COVID-19 patients for early assessment of the clinical course

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towards critical stage so that to improvise the recovery rate. It is suggested that a stringent serial monitoring of routinely investigated serum biomarkers and blood counts could predict vulnerability of the patient towards severity so that they can be kept under closed surveillance. Large scale prospective studies may be designed to evaluate the laboratory and clinical parameters in mild, moderate and severe cases so as to prepare an algorithm for better assessment and management of COVID-19 cases.

SUMMARY POINTS

- Number of males are more than three times than females in severe COVID-19 cases
- Probability of death was nearly 12 times in severe cases than non-severe cases
- Primary health centers lack facilities for specialized parameters, thus close monitoring of routinely investigated parameters would aid clinicians to predict the course of the disease
- Besides inflammatory markers, serum urea, liver enzymes, NLR, and ESR are elevated in severe form of the disease
- Lymphopenia, eosinopenia and hypoalbuminemia were found to increase the duration of hospital stay
- Instead of single marker CRP, a combination of serum analytes such as CAR would be a better marker for predicting prognosis

AUTHOR CONTRIBUTIONS

S.D. Prasad and A.K. Behera designed the study, performed the statistical analysis. S. Patel and R. Nanda wrote the protocol and wrote the first draft of the manuscript. S.D. Prasad and S. Patel managed the analyses of the study. E. Mohapatra managed the literature searches and intellectual content. All authors read and approved the final manuscript.

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DECLARATION OF INTERESTS

The authors declare that there was no conflict of interest.

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Abbreviations

Alb: Albumin (Gm/dL)
ALP: Alkaline phosphatase (U/L)
ALT: Alanine transaminase (U/L)
A/G: Albumin-to-Globulin ratio
AST: Aspartate transaminase (U/L)
AST/ALT: AST-to-ALT ratio
AUC: Area under curve
BC: Basophil count (%)
C3: Complement factor C3 (mg/dL)
C4: Complement factor 4 (mg/dL)
C3/C4: C3-to-C4 ratio
Ca: Calcium total (mg/dL)
CAR: CRP-to-Albumin ratio
CBC: Complete blood count
CI95%: Confidence interval of 95%
Cl^-: Chloride ion (mmol/L)
COVID-19: Coronavirus disease of 2019
Cr: Creatinine (mg/dL)
CRP: C-Reactive protein (mg/dL)
CS: COVID-Score
DBil: Direct bilirubin (mg/dL)
DM: Diabetes mellitus
EC: Eosinophil count (%)
ESR: Erythrocyte sedimentation rate (mm)
Fer: Ferritin (ng/ml)
GGT: Gamma glutamyl transferase (U/L)
Hb: Hemoglobin (Gm/dL)
Hct: hematocrit (%)
HS: Hospital stay (days)
IgG: Immunoglobulin G (mg/dL)
IgM: Immunoglobulin M (mg/dL)
IgG/IgM: IgG-to-IgM ratio
INR: International normalized ratio
K^+: Potassium ion (mmol/L)
LC: Lymphocyte count (%)
LDH: Lactate dehydrogenase (U/L)
LMR: Lymphocyte-to-monocyte ratio
MC: Monocyte count (%)
MCH: Mean corpuscular hemoglobin (pg)
MCHC: Mean corpuscular hemoglobin concentration (Gm/dL)
MCV: Mean corpuscular volume (fL)
Mg: Magnesium (mg/dL)
Na^+: Sodium ion (mmol/L)
NC: Neutrophil count (%)
NLR: Neutrophil-to-lymphocyte ratio
OR: Odds ratio
PC: Platelet count ($x10^3$/L)
Phos: Phosphorous (mg/dL)
PT: Prothrombin time (sec)
RBC: Red blood cell count ($x10^6$/L)
RDW: Red cell distribution width (%)
ROC: Receiver operating characteristics
SARS-CoV-2: Severe acute respiratory syndrome coronavirus-2
SD: Standard deviation
SE: Standard error
SpO_2: Oxygen saturation (%)
TBil: Total bilirubin (mg/dL)
TLC: Total leucocyte count ($x10^3$/L)
TP: Total protein (Gm/dL)
UA: Uric acid (mg/dL)
Ur: Urea (mg/dL)
WHO: World Health Organization