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Original Article

Biochemistry Section

Predictors of Severity and Mortality among Hospitalised COVID-19 Patients during First Three Waves- A Retrospective Study

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ABSTRACT

Introduction: Coronavirus disease 2019 (COVID-19) was was a global pandemic outbreak that emerged in three waves of varying severity. The inflammatory markers can be used to identify the transition of severity of the disease from a mild to severe or critical illness and also to predict the mortality. To improve clinical outcomes, continuous monitoring of these biomarkers in hospitalised patients is essential.

Aim: To compare the serum C-Reactive Protein (CRP), Lactate Dehydrogenase (LDH), serum ferritin and D-Dimer as a predictor of severity and mortality in hospitalised patients in different clinical categories during three waves of COVID-19 .

Materials and Methods: This retrospective study was conducted at Vydehi Institute of Medical Sciences and Research Centre, Karnataka. A total of 6189 patient's data were retrieved from medical record section with confirmed cases of COVID-19 admitted during the three waves of COVID-19. The results of four inflammatory marker on the day of admission were analysed in mild, moderate, severe and critically ill patients and compared with disease severity, survival and death and also assessed the difference between first, second, and third wave of COVID-19 for sensitivity and specificity. Data was analysed by Chi-square test, Mann Whitney U test, ANOVA (Analysis of Variance), and Kruskal Wallis test and Receiver Operating Curve (ROC).

Results: The median age of patients in the mild category was found to be younger in all three waves compared to those in the severe and critically ill categories. In terms of gender distribution, males were more prevalent than females. Inflammatory markers were significantly increased in all three waves in the severe and critically ill categories. The median CRP (mg/dL) values were significantly increased in the critically ill in wave three compared to waves one and two. The median values of LDH IU/L significantly increased in critically ill patients in the second wave. The median values of ferritin increased significantly in wave one. The D-dimer values significantly increased in the critically ill category in wave three.

Conclusion: Before proceeding on to a specific diagnosis by RT-PCR, a combination of standard laboratory markers (CRP, LDH, and Ferritin D-Dimer) could accurately and reliably predict the diagnosis of COVID-19 with established sensitivity and specificity.

Keywords: Coronavirus disease-2019, Inflammation, Pandemic, Lactate dehydrogenase

INTRODUCTION

Coronavirus disease 2019 (COVID-19), caused by Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) was declared a pandemic by the World Health Organisation (WHO) on March 11, 2020 [1]. COVID-19 presents with varying degree of clinical severity, ranging from asymptomatic to severe pneumonia, Acute Respiratory Distress Syndrome (ARDS), and even death [2]. Development of inflammatory reactions in patients with COVID-19 occurs due to rapid viral replications of SARS-CoV-2 [3].

Progressive cellular destruction leads to accumulation of macrophages and monocytes, which induces the release of cytokines and chemokine resulting in hyperinflammatory state called cytokine storm [4,5]. Cytokine storms presents clinically with progressive deterioration characterised by coagulopathy, hypotension, and multiple end-organ dysfunction [6]. A number of inflammatory biomarkers had the potential to monitor and determine COVID-19 infection severity and fatality. Patients with COVID-19 had previously been found to have elevated levels of inflammatory markers like CRP and ferritin, LDH as well as alterations like D-Dimer, but little was known regarding their relationship with disease severity among three waves of COVID-19 pandemic [7]. Computed Tomography (CT) imaging and chest X-ray, also plays an important role in assessing the severity of the disease but has got its own limitations of cost, in ability to use during high oxygen requirement or ventilator support [8].

Improved therapeutic outcomes may be associated to anti-inflammatory treatments designed to reduce these cytokine levels. The first COVID-19 infections in India were reported on 30th January, 2020 in three places in Kerala, and they persisted until September 2020 [9]. A second wave begins in March 2021, which was much more severe than the first, with limited vaccines, hospital beds, oxygen supports and other medical services in parts of the country and it lasted till December 2021 [10]. The third wave in India started in the third week of November 2021 and peaked in Jan 2022 [11]. The similarities and differences between the characteristics of the three waves remain largely unknown, change in variants pathogenesis and transmission among three waves was essential.

Thus ongoing pandemic would benefit from the most efficient distribution of limited human and technical resource such as oxygen cylinder, ICU admission, treatment modalites and vaccines if routine laboratory parameters were analysed and established to assess the severity of the disease among three waves. Early identification of patients who were predicted to deteriorate was crucial for the appropriate utilisation of the available medical resources in each waves of pandemic. At present many literature is available only on altered inflammatory markers in first and second wave of COVID-19

[12,13]. Few studies have been conducted in the emergency unit regarding the study of early routine biomarkers for the prediction of morbidity and mortality of COVID-19 patients [14-17]. But, there was no article available in Indian setup, comparing the alteration in inflammatory markers in all three waves of COVID-19.

Hence, present study was conducted to understand the utility of inflammatory markers in each wave and also could be identified as selective biomarkers during different clinical staging. Present study was undertaken to establish the link between the trend of proinflammatory cytokines, such as CRP, LDH, ferritin, and D-Dimer and the overall outcomes in patients with COVID-19 in terms of survival and mortality in three waves.

MATERIALS AND METHODS

A retrospective study was conducted at a tertiary care centre for the care of COVID-19, Vydehi Institute of Medical Sciences and Research Centre, Karnataka, India. A comparative data analysis was done with COVID-19 patients who were hospitalised during the first wave from 01-03-2020 to 01-05-2020, the second wave from 1-10-2020 onwards till 01-05-2021 and third wave from 01-11-2021 to 01-03-2022. The study protocol was approved by the Ethics Committee (VIEC: /2021/APP/004, EC reg NO: ECR/747/Inst/KA/2015/RR-18), and a waiver for consent was granted from all the study subjects.

Inclusion criteria: The patients aged ≥18 years of both gender who were hospitalised with a positive SARS-CoV-2 Polymerase Chain Reaction (PCR) assay of nasal, pharyngeal, or lower respiratory tract samples were included.

Exclusion criteria: Patients with co-morbid conditions such as heart attacks, trauma, infections, burns, chronic inflammatory diseases like lupus, vasculitis, rheumatoid arthritis and inflammatory bowel diseases were excluded from the study. Patients with suspected COVID-19 infection without laboratory confirmation by RT-PCR and those who presented to the hospital with symptoms consistent with COVID-19 but did not required hospitalisation, were also excluded.

Data collection: A thorough computerised search from the medical record section was conducted based on accessibility, demographic information, and results of laboratory inflammatory results like serum CRP, LDH, Ferritin, and D-Dimer levels within 24 hours of hospitalisation, clinical state, requirement for oxygen, and ventilatory support. The data was retrieved in all three waves and sorted. Patients with only serum CRP data were collected in wave one (N=1729), wave two (N=458), and wave three (N = 52). LDH wave one N (1398). wave two (N=448), wave three has (N=11). Ferritin wave one (N=1683) wave two (N=410), wave three-nil data. D-Dimer wave one (N=1702), wave two (N=25) and wave three (N=52).

The disease severity at the time of hospitalisation was classified based on the new guidelines for COVID-19 disease severity by National Institute of Health issued by the Government of India as described below [18]. Inflammatory marker values were recorded in mild, moderate, severe and critically ill categories [19] and compared in all the 3 waves.

Mild: Individuals who had any of the various signs and symptoms of COVID-19 (e.g., fever, cough, sore throat, malaise, headache, muscle pain, nausea, vomiting, diarrhea, loss of taste and smell) but who do not have shortness of breath, dyspnoea, or abnormal chest imaging.

Moderate: Individuals who show evidence of lower respiratory disease during clinical assessment or imaging and who have an oxygen saturation (SpO2) of 94% on room air at sea level.

Severe: Individuals with a SpO2 of 94% on room air at sea level, a PaO_2/FiO_2 [oxygen in arterial blood (PaO_2) to the fraction of the oxygen in the inspired air (FiO_2)]. ratio of 300 mmHg, a respiratory rate greater than 30 breaths per minute, or lung infiltrates greater than 50%. Critically ill-Individuals who had respiratory failure, septic shock, and/or multiple organ dysfunction [19].

CRP was measured using the Nephalometric method in the Beckman image 800.range for CRP is 0.07-0.75 mg/dL. A Beckman dxc- 860 chemistry analyser was used to measure LDH and ferritin. LDH-100-190 IU/L below 60 years, above 60 years, 110-210 IU/L. Ferritin-20-250 ng/mL, D-Dimer analysed by ACL elite PRO coagulation analyser with reference range of < 255 ng/mL [20].

STATISTICAL ANALYSIS

Data entry was done in a Microsoft Excel 2007 spreadsheet. Data analysis was carried out by IBM Statistical Package for Social Sciences version 25.0 software. All p-values <0.05 were deemed statistically significant. Data was presented as either median or interquartile range for continuous, or frequency and percentages for categorical variables. The normality of the data was checked through the Shapiro-Wilk test. A non parametric test, i.e., the Mann Whitney U test, was used to compare differences between two independent groups when the data was not normally distributed. The Chi-square test, or Fisher's exact test, was applied to the frequency distribution accordingly. A Receiver Operating Characteristic (ROC) curve was drawn to identify the optimal cut-off points of biomarkers to know their prognostic value. The Kruskal-Walli test was performed separately for waves 1, 2, and 3 to compare the median values of age, sex, and CRP/LDH/Ferritin/D-Dimer between the different severity grades. The Mann Whitney test was performed as a post hoc test to check the intragroup significance.

RESULTS

In all the three waves median age of patients in mild category found to be younger compared to moderate, severe and critically ill. According to genderwise distribution, male patients were more compared to females but this difference was not statistically significant. The median and IQR value of CRP (mg/dL) increased significantly in all three waves from mild to critically ill category. In wave one 1619 (93.63%) were survived and 110 (6.3%) had mortality. In wave two 392 (85.58%) survived and 66 (14.4%) had mortality. In wave three CRP (mg/dL) significantly increased in critically ill above 8.15 (2.92, 25.90)compared to other two waves and with no mortality [Table/Fig-1].

In wave one, AUC of 0.69 with cut-off points of CRP 1.25 mg/dL (12.5 mg/L) suggest it as a better marker of mortality in critically ill category (sensitivity-71.3%, specificity-59.8%). In wave two: AUC of 0.67 with cut-off points of CRP 4.16 mg/dL (41.6 mg/L) suggests it as a better marker of mortality in severe category (sensitivity-70.10%, specificity-56%). In wave three: AUC of 0.764 with cut-off points of CRP 1.92 mg/dL (19.2 mg/L) suggests it as a better marker of mortality in severe category (sensitivity-75%, specificity-42.1%) [Table/Fig-2].

The median age of patients in the mild category was found to be younger in all three waves compared to those in the moderately severe and critically ill categories. Males outnumbered females in terms of gender distribution, but the difference was not statistically significant. LDH (IU/L) median and IQR values increased significantly across all three waves, from mild to critically ill. In wave one, LDH (IU/L) significantly increased in critically ill patients above 380 (280.5, 511.5); 1272 (90.98%) patients survived, and 126 (9.01%) patients died. The number of severely and critically ill patients increased significantly in wave two 253.5 (197.2, 321.2) and 426.5 (299.5, 613.2), respectively, with 376 (83.92%) patients surviving and 72 (16.07%) dying. In wave three, LDH (IU/L) significantly increased in critically ill patients above 369 (207,522) with no mortality [Table/Fig-3].

In wave one, AUC of 0.63 with cut-off points of LDH 204IU/L suggests it as a better marker of mortality in critically ill category (sensitivity-73%, specificity-53.8%). In wave two, AUC 0.84 with cut-off points of LDH was 226.5IU/ better marker of mortality in severe category (sensitivity - 90%, specificity- 63.5%). In wave three,

	Wave one (N=1729)					Wave two (N=458)					Wave three (N=52)				
Variables	Mild	Moderate	Severe	Critical	p-value	Mild	Moderate	Severe	Critical	p-value	Mild	Moderate	Severe	Critical	p-value
Age (Median and IQR)	38 (27, 53)	57 (40, 66)	51 (40, 64)	57 (46, 67)	<0.001	44 (33, 55)	55 (44, 65)	53 (40.2, 62.5)	55 (45, 65)	<0.001	43 (22,51)	38 (27,64)	64 (53,71)	55 (52,73)	<0.001
CRP (mg/dL)	0.62 (0.26, 1.81)	1.13 (0.46, 4.48)	3.67 (0.97, 8.95)	7.49 (2.32, 15.2)	<0.001	2.71 (0.58, 7.53)	2.4 (1.0, 8.52)	6.03 (2.23, 10.60)	7.59 (2.97, 12.80)	<0.001	2 (0.47, 3.58)	0.89 (0.52, 6.48)	3.17 (1.68, 10.44)	8.15 (2.92, 25.90)	<0.001
Female (n, %)	350 (32.1)	8 (27.6)	132 (33.3)	67 (34.9)	0.79	75 (31.3)	16 (34)	36 (34.6)	19 (28.4)	0.82	3 (42.9)	5 (31.2)	5 (31.2)	6 (50.0)	0.213
Male (n, %)	752 (67.9)	21 (72.4)	272 (66.7)	127 (65.1)		165 (68.8)	31 (66)	68 (65.4)	48 (71.6)		4 (57.1)	12 (68.8)	11 (68.8)	6 (50.0)	
Alive (n, %)	1075 (98)	27 (93.1)	388 (95.1)	129 (66.2)	<0.001	227 (94.6)	45 (95.7)	84 (80.8)	36 (53.7)	<0.001	7 (100)	19 (/100)	12 (100)	14 (100)	
Death (n, %)	22 (2)	2 (6.9)	20 (4.9)	66 (33.8)		13 (5.4)	2 (4.3)	20 (19.2)	31 (46.3)		-	-	-	-	NA

[Table/Fig-1]: Comparison of CRP level, demographic characteristics among stages of COVID-19 diseases in wave one, two and three.
Data were presented as median (inter quartile range) for continuous variable

CRP	Stages	Sensitivity	Specificity	Cut-off	AUC	95% CI
Wave one	Moderate	62.10	42.40	0.76	0.51	0.41, 0.60
	Severe	72.30	23.8	0.45	0.45	0.37, 0.53
	Critically ill	71.3	59.8	1.25	0.69	0.66, 0.72
Wave two	Moderate	63.50	52.00	3.43	60.8	0.55, 0.66
	Severe	72.3	70.2	2.53	0.77	0.73, 0.80
	Critically ill	70.10	56	4.16	0.67	0.60, 0.73
Wave three	Moderate	60.7	47.1	1.15	0.42	0.262, 0.58
	Severe	75	42.1	1.92	0.764	0.499, 0.805
	Critically ill	71.4	20.3	0.764	4.48	0.606, 0.921

[Table/Fig-2]: Area under curve, optimal probability cut-off, sensitivity, and specificity of CRP in predicting mortality in wave one, two and three.

In wave two, median age of patients in mild category found to be younger compared to moderate, severe and critically ill above 51 years to 57 years. Males outnumbered females in terms of gender distribution, but the difference was not statistically significant. The median and IQR value of ferritin (ng/mL) increased significantly in two waves from mild to critically ill category. In wave one and two median and IQR value of Ferritin (ng/mL) significantly increased in severe and critically ill above 339 (154.6, 586.2), 466.6 (218.9, 881.2), 325.8 (146, 577.2), 424.1 (197.1, 842.4) respectively.

In wave one, 1556 (92.5%) patients survived and 127 (7.5%) patients had mortality, with p-values <0.001. In wave two, 350 (85.4%) patients survived and while 60 (14.6%) died [Table/Fig-5].

	Wave one (N=1398)					Wave two (N=448)					Wave three (N=11)				
Variables	Mild	Moderate	Severe	Critical	p-value	Mild	Moderate	Severe	Critical	p-value	Mild	Moderate	Severe	Critical	p-value
Age (in years)	40 (29, 54)	56 (40, 65.5)	51 (41, 63.7)	58 (46, 67.5)	<0.001	45 (33.2, 56)	54.5 (41.2, 64.2)	52.5 (39, 63)	55 (45.2, 65)	<0.001	51 (20,51)	60 (29,64)	71 (68,71)	55 (53,78)	0.231
LDH (IU/L)	181 (153, 229)	177 (161.2, 251.2)	246 (199.2, 320.5)	380 (280.5, 511.5)	<0.001	240.5 (187, 320)	253.5 (197.2, 321.2)	328 (246.5, 451)	426.5 (299.5, 613.2)	<0.001	161 (158, 299)	167 (153, 263)	272 (175, 213)	369 (207, 522)	0.147
Male n (%)	263 (32.3)	6 (27.3)	115 (32.7)	66 (31.6)	0.95	74 (32.5)	16 (34.8)	34 (32.1)	17 (25)	0.64	2 (66.7)	3 (100)	2 (66.7)	1 (33.3)	0.836
Female n (%)	552 (67.7)	16 (72.7)	237 (67.3)	143 (68.4)		154 (67.5)	30 (65.2)	72 (67.9)	51 (75)		1 (33.3)	0 (0)	1 (33.3 (	2 (66.7)	
Alive n (%)	794 (97.4)	20 (90.9)	329 (93.5)	129 (61.7)	<0.001	216 (94.7)	44 (95.7)	84 (79.2)	32 (47.1)	<0.001	3 (100)	3 (100)	3 (100)	3 (100)	NA
Death n (%)	21 (2.6)	2(9.1)	23 (6.5)	80 (38.3)		12 (5.3)	2 (4.3)	22 (20.8)	36 (52.9)		-	-	-	-	

[Table/Fig-3]: Comparison of LDH level, demographic characteristics among stages of COVID-19 diseases in wave one, two and three.

AUC=0.86 with cut-off points of LDH was 293.5 IU/L better marker of mortality in critically ill category (sensitivity-60%, specificity-11%) [Table/Fig-4].

LDH	Stages	Sensitivity	Specificity	Cut-off	AUC	95% CI
Wave one	Moderate	45.5	40.1	191.5	0.42	0.31, 0.52
	Severe	47.8	43.8	258.5	0.41	0.34, 0.49
	Critically ill	73	53.5	204.5	0.63	0.60, 0.67
Wave two	Moderate	73.6	50.3	258.5	0.629	0.57, 0.68
	Severe	90	63.5	226.5	0.84	0.82, 0.87
	Critically ill	80.9	51.8	270.5	0.75	0.69, 0.81
Wave three	Moderate	71.4	60.2	164	0.259	0.00, 0.585
	Severe	66	20	267	0.667	0.034, 0.986
	Critically ill	60	11	293.5	0.852	0.557, 1

[Table/Fig-4]: Area under curve, optimal probability cut-off, sensitivity, and specificity of LDH in predicting mortality in wave one, two and three.

In wave one, AUC of 0.67 with cut-off points of ferritin 187.6 (ng/mL) suggests it as a better marker of mortality in critically ill category (sensitivity-70%, specificity-57.3%), In wave two, AUC 0.74, 0.61 with cut-off points of Ferritin 183.9/301.5 (ng/mL) suggests it as a better marker of mortality in severe and critically ill category (sensitivity-80.1%/58.1, specificity - 54.2/55.1%) [Table/Fig-6].

The median age of patients in the mild category was found to be younger in all three waves compared to those in the moderately severe and critically ill categories. Males outnumbered females in terms of gender distribution, but the difference was not statistically significant. The median and IQR values of D-Dimer ng/mL increased significantly in all three waves, from mild to critically ill. In wave one, the median and IQR values of D-Dimer significantly increased in severe and critically ill patients with 287 (213, 587), 462 (300, 1094.5), and 1583 (93%) patients surviving, while 119 (6.99%) patients died. In wave two, the median and IQR values of D-Dimer (ng/mL) significantly increased in the severe and critically ill categories 408.5 (291,861), 584 (290.5,

	Wave one (N=1683)					Wave two (N=410)					Wave three (nil)
Variables	Mild	Moderate	Severe	Critical	p-value	Mild	Moderate	Severe	Critical	p-value	
Age (in years)	39 (28, 53)	56 (38.7, 65.5)	51 (41, 64)	57.5 (46.2, 67)	<0.001	45 (35, 55)	55 (43, 65)	52 (40, 62.5)	55 (45, 64.2)	<0.001	*
Ferritin (ng/mL)	120.1 (50.1, 263.5)	122.3 (62.6, 233.2)	339 (154.6, 586.2)	466.6 (218.9, 881.2)	<0.001	217.6 (103, 540.6)	229.4 (138.4, 450.6)	325.8 (146, 577.2)	424.1 (197.1, 842.4)	0.006	*
Female (n, %)	327 (31.6)	8 (30.8)	133 (32.7)	72 (33.3)	0.95	65 (31.4)	15 (33.3)	33 (34.4)	16 (25.8)	0.71	*
Male (n, %)	707 (68.4)	18 (69.2)	274 (67.3)	144 (66.7)		142 (68.6)	30 (66.7)	63 (65.6)	46 (74.2)		
Alive (n, %)	1011 (97.8)	24 (92.3)	384 (94.3)	137 (63.4)	<0.001	193 (93.2)	43 (95.6)	80 (83.3)	34 (54.8)	<0.001	*
Death (n, %)	23 (2.2)	2 (7.7)	23 (5.7)	79 (36.6)		14 (6.8)	2 (4.4)	16 (16.7)	28 (45.2)		

[Table/Fig-5]: Comparison of ferritin level, demographic characteristics among stages of COVID-19 diseases in wave one, two and three.

Ferritin	Stages	Sensitivity	Specificity	Cut-off	AUC	95% CI
Wave one	Moderate	53.8	37.6	121.30	0.39	0.29, 0.49
	Severe	71.1	37.8	167.5	0.48	0.39, 0.56
	Critically ill	70	57.3	187.6	0.67	0.64, 0.70
Wave two	Moderate	53.1	54.8	287.3	0.46	0.47, 0.60
	Severe	80.1	54.2	183.9	0.74	0.70,0.77
	Critically ill	58.1	55.7	301.5	0.61	0.54,0.69
Wave three	Nil					

[Table/Fig-6]: Area under curve, optimal probability cut-off, sensitivity, and specificity of Ferritin in predicting mortality in wave one, two and three.

973), respectively, and no mortality. In wave three, D-Dimer significantly increased in all clinical categories, significantly in the critically ill above 795 (233, 1200), and with no mortality [Table/Fig-7].

in severe and critically ill category (sensitivity-90.2%, specificity-60.5%) [Table/Fig-8].

DISCUSSION

The COVID-19 pandemic had transformed into a worldwide disaster, marked by a significant incidence of complications, mortality, and even economic disruption. Accordingly, there is a critical need for affordable and feasible markers to streamline the diagnostic process and quantify the severity of the condition. The increased serum levels of CRP, LDH, D-dimer, and Ferritin at the time of admission within 24 hours in COVID-19 patients indicated of early ICU admission and categorisation of severity and requirement of an early treatment in order to avoid predicted mortality.

First retrospective study in India in which increased inflammatory markers such as serum levels of CRP, LDH, D-dimer, and Ferritin

	Wave one (N=1702)					Wave two (N=25)					Wave three (N=52)				
Variables	Mild	Moderate	Severe	Critical	p-value	Mild	Moderate	Severe	Critical	p-value	Mild	Moderate	Severe	Critical	p-value
Age (in years)	38 (28, 53)	57 (40, 67)	51 (41,64)	57 (46,67)	<0.001	48 (34.5, 63)	NA	47 (44.7, 53.5)	46 (36,63.5)	0.95	46 (32,51)	29 (27,64)	62 (49, 71)	54 (49, 73)	<0.001
D-Dimer (ng/mL)	213.5 (182)	244.5 (206.7, 305.7)	287 (213, 587.2)	462 (300, 1094.5)	<0.001	261.5 (232.5, 293.7)	NA	408.5 (291, 861.7)	584 (290.5, 973)	0.01	523 (281, 1655)	538 (448, 1078)	579 (403, 771)	795 (233, 1200)	<0.001
Female (n, %)	339 (32.1)	9 (30)	137 (33.6)	68 (32.7)	0.94	2 (20)	NA	1 (16.7)	0	0.43	6 (75)	14 (73.7)	6 (54.5)	4 (28.6)	<0.001
Male (n, %)	717 (67.9)	21 (70)	271 (66.4)	140 (67.3)		8 (80)	NA	5 (83.3)	9 (100)	-	2 (25)	5 (26.3)	5 (45.5)	10 (71.4)	0.906
Alive (n, %)	1038 (98.3)	28 (93.3)	385 (94.4)	132 (63.5)	<0.001		NA	6 (100)	9 (100)	NA	8 (100)	19 (100)	11 (100)	14 (100)	<0.001
Death	18 (1.7)	2 (6.7)	23 (5.6)	76 (36.5)			NA	0	0	-	-	-	-	-	NA

[Table/Fig-7]: Comparison of D-dimer level, demographic characteristics among stages of COVID-19 diseases in wave one, two and three.

In wave one, AUC of 0.75 with cut-off points of D-Dimer 240.5 ng/mL suggests it as a better marker of mortality in critically ill category (sensitivity-85.1%, specificity-54.8%), In wave two, AUC (0.76) with cut-off points of D-Dimer 310 ng/mL suggest it as a better marker of mortality in critically ill category (sensitivity-66.7%, specificity- 62.5%), In wave three, AUC (0.45) with cut-off points of D-Dimer 302 ng/mL suggest it as a better marker of mortality

D-Dimer	Stages	Sensitivity	Specificity	Cut-off	AUC	95% CI
Wave one	Moderate	50	51.3	245.5	0.5	0.42, 0.58
	Severe	63.7	54.2	240.5	0.6	0.57, 0.63
	Critically ill	85.1	54.8	240.5	0.75	0.72, 0.79
Wave two	Moderate	NA				
	Severe	66.7	68.4	340	0.64	0.38, 0.89
	Critically ill	66.7	62.5	310	0.76	0.56, 0.94
Wave three	Moderate	80	50.1	352	0.48	0.325, 0.651
	Severe	90.2	60.5	302	0.450	0.284, 0.617
	Critical	75.2	55.1	459	0.562	0.362, 0.7

[Table/Fig-8]: Area under curve, optimal probability cut-off, sensitivity, and specificity of D-Dimer in predicting mortality in wave One-two and three.

extensively analysed for age, sex mortality and survivor in clinical severity individually. As per data available more number of subjects were found in wave 1 compared to 2nd/3rd waves. The reason behind the difference between the two waves was not exactly known. The probable explanation suggests that a new variant of SARS-CoV-2 emerged in second and third wave with less virulence, better knowledge about disease state, early laboratory COVID-19 testing and availability of well equipped intensive care units with ventilators for critical severity, better treatment modality and finally initiation of vaccination campaign which provided immunity before third wave making COVID 19 infection less severe [21].

Comparing the age-wise percentage distribution of cases during both 1st, 2nd and 3rd the waves, in all three waves more or less same and it was attributed that the percentage distribution of median age of the cases during the first wave and second was above 50 to 55 years in severe and critically ill, while in the third waves in critically ill above 60 years which was in line with study of Jain VK et al., and Han Y et al., in their study suggested older subjects were infected in the first wave and younger population in the second [22,23]. Present study was also in line with Singh S et al., suggested as compared to the first and third waves, the cases

during the second wave of the pandemic presented at a younger age group [24].

The SARS-CoV-2 virus could affect both genders, but many studies done across the world showed that older males were more (> 50%) susceptible. Probable mechanism for male susceptibility was that alteration in innate immunity due to hormonal response elements like putative Androgen Response Elements (AREs), Oestrogen Response Elements (ORE). Men probably smoke more frequently and drink more heavily than women, which was also linked to chronic diseases. Women also typically have better immune systems than men [24-28]. In present study in all three waves male subjects were more compared to females, finding were in line with above studies.

In the present study, inflammatory markers such as CRP, LDH, D-Dimer and Ferritin were found to be significantly higher in severe and critically ill patients in all three waves. This demonstrates that patients with severe/ critical illness who were hospitalised in the first wave had more severe disease compared with the other two waves.

Study conducted by Pan F et al., concluded that inflammatory parameter CRP >77.35 mg/L, LDH >481 U/L, and D-dimer >3.06 mg/L were increased in patients in severe and critically ill clinical category and associated with poor prognosis, multiorgan failure and death which was similar to the present study [29]. Thus, elevated CRP was linked with both severity and mortality in COVID-19 patients, suggestive of prominent hyperinflamation. Serum ferritin was an acute-phase protein and LDH which could be used as a prognostic marker for tissue damage it was suggested that hyperferritinemia in COVID-19 patients was most likely due to the cytokine storm. Thus elevated ferritin levels had been associated not only to inflammation, but also to direct cellular damage and more organ dysfunction. Since LDH was expressed in lung tissue (isozyme 3), patients with severe COVID-19 infections could be predicted to release higher levels of LDH in the circulation, as a severe form of interstitial pneumonia [30].

Ceci FM et al., [31] in their study where results were analysed by ANOVA, data revealed that LDH, Ferritin, CRP, and D-dimer levels were markedly elevated within 24 hrs of admission in emergency department linked with development of a worst course similar results observed in present study. Tan C et al., the AUC scores suggest that elevations in a combination of blood parameters (LDH, MGB, CPR, Ferritin and D-dimer) at the emergency section level could lead to severe (ICU and/or death) health outcomes [32].

CRP had an AUC of 0.69, 0.77, and 0.764. LDH (0.63, 0.84, 0.85) and ferritin (0.67, 0.61) [Table/Fig-2,4,6,8] were about 0.7, which was similar to the study done by Mardani R et al., who reported an AUC of 0.8 for CRP and LDH, which was in line with the present study [33]. The results suggested that CRP and LDH had the highest sensitivity and specificity in the critically ill and severe categories. A cut-off value of 301.5 ng/mL for ferritin in a two waves was close to the value (304 ng/mL) reported by Tular O et al., [34]. For LDH, the cut-off value was found to be 204.5-293.5 IU/L; this value was similar to the result (277 mg/dL) reported by Li C et al., [35]. For CRP, a cut-off value of 1.25 to 2.53 mg/dL was observed to be concomitant with a specificity of 0.77 and a sensitivity of 0.56, these findings were in line with those reported by Cheng B et al., [36].

Guan WJ et al., reported in their study that severe patients had a significantly higher level of D-dimer than non severe patients after analysing 1,099 patients with laboratory confirmed COVID-19 from over 550 hospitals in China [37]. D-Dimer levels were significantly higher in the critically and severely ill. COVID-19 patients. It was found that elevated D-dimer at admission was a risk factor for the death of adult patients [38]. Similar results were found in the study. The underlying mechanism was unknown in the relationship between elevated D-dimer and COVID-19 mortality. Thrombus formation and mortality were caused by a hyperinflammtory state with cytokine strom. Thus, if D-dimer measurements and monitoring were performed, COVID-19 mortality could be predicted.

More patients with COVID-19 were hospitalised in the first wave, with high mortality rate compared to a lower mortality rate, observed in the second and third wave. Contou D et al., [39], concluded that the difference in mortality between the first and other two waves was attributed to delay testing, a poor understanding about the infection, increased pathogenicity of COVID-19, a limitation of oxygen supply, Intensive Care Unit (ICU) admission, and needing mechanical ventilation failed non invasive respiratory support.

In the first wave, the treatment recommended was steroids as part of the recovery trial and increased length of hospital stay [39]. Because steroids, remdesivir, and convalescent plasma were used substantially more frequently in the second and third wave than in the first, mortality in the second wave was lower than in the first. In the second wave, all our patients had already had steroids administered before ICU admission; in third wave of COVID-19 with the initiation of vaccination in India hospitalisation and mortality observed only in severe and critically ill. Mild moderate cases treated at outpatient basis.

To summarise hyperinflammatory state, cytokine strom and mortality in COVID-19 with laboratory biomarkers well correlated with elderly aged subjects, male gender, and higher mortality in wave one. The outcomes were worse in first wave compared to second wave, followed by third wave.

Limitation(s)

Study had few limitations, such as the serum CRP, LDH, Ferritin and D-Dimer level were measured within the first 24 hours of hospitalisation, length of stay, treatment modality and iimmunisation factors were not considered.

CONCLUSION(S)

Inflammatory markers elevated in all three waves, based on sensitivity and specificity CRP remained as good marker of severity and mortality in all three waves suggestive of hyper inflammatory state, LDH and ferritin marker of severity in wave two suggestive sudden surge of cytokine storm due to rapid transmission and multi organ damage. D-Dimer found to better marker of severity and mortality in wave one due to hypercoagulable state. Thus inflammatory parameters predict clinical severity and which guides the early treatment.

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