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Comparative retrospective study of biochemical and haematological biomarkers and their alterations in First and Second COVID-19 wave in a tertiary care facility in Ghaziabad

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Abstract---Background: The second wave of COVID-19 in India started in the middle of March 2021. In comparison to the first wave, the second wave of COVID-19 was characterized by a massive increase in the number of cases, reduced supplies of essential treatments, and increased deaths, particularly in the young population. In addition, the second wave was also characterized by the reporting of numerous cases of mucormycosis. Surprisingly, it was the younger population who were more affected in the second wave of the disease. The distinct difference in the first and second waves of COVID-19 in India makes it imperative to study the biochemical and pathological differences between the two. Methods: We aimed to assess the biochemical and pathological biomarkers and their alterations in 1st and 2nd waves of COVID-19 in a tertiary care L3 facility in Ghaziabad. Reports of haematological and biochemical parameters of the admitted patients were compiled retrospectively for statistical analysis based on the severity status of the patient and were compared between the first wave and second wave of COVID-19. Results: Our study observed PCV and sodium to be significantly different between different grades of

severity of COVID-19 in the first wave whereas total leukocyte count, total protein, and globulin in the second wave. The liver enzymes i.e. SGOT and SGPT were significantly elevated in the second wave when compared with the first wave in all the grades of severity of COVID-19. Conclusion: Our study concluded that the second wave of COVID-19 has affected liver function more severely than the first wave in Ghaziabad. Total leukocyte count has emerged as a better marker for distinguishing severe cases from mild cases in the second wave of COVID-19.

Keywords--COVID-19, first wave, second wave, biochemical biomarkers, hematological biomarkers.

Introduction

The second wave of COVID-19 in India started in the middle of March 2021 [1]. In comparison to the first wave, the second wave of COVID-19 was characterized by a massive increase in the number of cases, reduced supplies of essential treatments, and increased deaths particularly in the young population [2]. The intensity of the second wave was attributed to the emergence of novel strains of the SARS CoV-2 virus, poor adherence to containment strategies, and a large number of the vulnerable population [3]. In addition, the second wave was also characterized by the reporting of numerous cases of mucormycosis [4]. Surprisingly, it was the younger population who were more affected in the second wave of the disease [4]. The various factors that accentuated the second wave of COVID-19 in India are hypothesized to be the interplay of mutant strains, violation of COVID appropriate behaviour, and government and public complacency on initiation of the vaccination drive [5]. Apart from the epidemiological characteristics, the first and second waves of COVID-19 also differed with respect to the laboratory parameters. When compared with the first wave, the second wave in Pakistan has less severe inflammatory markers [6]. Similarly, interleukin-6 and d-dimer concentrations were more severely altered in the first wave when compares with the second wave in the Spanish population [7]. However, the study conducted in the Turkish population revealed higher serum CRP levels in patients in the second wave when compared with those in the first wave [8]. The distinct difference in the first and second waves of COVID-19 in India makes it imperative to study the biochemical and pathological differences between the two. Such a study would throw light on the differences between first and second COVID-19 waves based on various hematological and biochemical parameters and would help in identifying those biomarkers that had higher efficacy in detecting severe cases of COVID-19 in both first and second waves of COVID-19. Hence we aimed to compare the biochemical and pathological biomarkers between the first and Second COVID-19 to identify similarities and differences in alterations in biomarkers between the first and Second COVID-19 waves in a tertiary care facility in Ghaziabad.

Materials and Methods

This retrospective study analyzed data from Santosh Hospital Ghaziabad, UP and a total of 356 patients with Covid-19 admitted from April 27, 2020, to September 15, 2020 (first wave) and 583 Covid -19 patients admitted from January 01, 2021, to June 17, 2022 (second wave). All patients were confirmed to have SARS-Cov-2 infection by reverse transcriptase-polymerase chain reaction (RT-PCR) test. Patients with missing laboratory test results data and in view of lack of complete clinical data were excluded from the study. The recruited patients were classified as Mild, Moderate, and Severe based on the Guidelines on Clinical Management of COVID – 19, Ministry of Health & Family Welfare, Government of India.

Variables were expressed as median and interquartile range (IQR) values, compared using Mann-Whitney U and Kruskal- Wallis test since data were non-normally distributed. For all the parameters: gender-wise (i.e. male/ Female), Severity wise (i.e. mild/ moderate/ severe) and wave wise (i.e. first/ second) comparison were done. To identify prognostic efficacy of parameters on the severity in wave first and wave second were done based on receiver operating characteristic (ROC) Analysis for which cut-off values for each parameter were established using the Youden index and the area under the ROC curve (AUROC), std. error and 95% CI were calculated.

Results

The data from 939 patients admitted to the hospital between April 27, 2020, and September 15, 2020 (first wave) and between January 01, 2021, and June 17, 2022 (second wave), meeting the inclusion criteria, were analyzed in our study. As shown in Table 1 significant differences were observed in parameters- Hemoglobin ($p<0.001$), RBC ($p=0.041$), PCV ($p=0.018$), and Creatinine ($p=0.0345$) between females and males in first wave of covid 19. But in the second wave for the parameters, no significant difference was observed between female and male patient's (Table 2).

Table 1
Laboratory findings of patients admitted in first wave of COVID-19

	Female (n=130)	Male (n=226)	P value
	Median (IQR)	Median (IQR)	
Haematological Parameters			
Hemoglobin(g/ dl)	11.1(10-12.2)	12.8(11.3-13.9)	<0.0001
Platelet	2.08 (1.5-2.9)	2.21(1.49-3.05)	0.5646
RBC	4.47(4.02-5.04)	4.22(3.83-4.8)	0.0414
PCV	38.6(34.5-43.7)	37.7(32.9-41.7)	0.0177
MCV	86.35(77-91.4)	86.2(81-91.3)	0.2563
MCH	29(26.9-30.7)	28.5(26.3-30)	0.3629
MCHC	32.8(32-33.4)	32.6(31.6-33.3)	0.2353
Total Leukocyte Count	6755(5440-10720)	7000(4970-10260)	0.6364
Liver Function Tests			

Total Bilirubin(mg/dl)	0.72(0.68-0.86)	0.72(0.68-0.86)	0.5079
Direct Bilirubin(mg/dl)	0.24(0.22-0.28)	0.24(0.22-0.27)	0.3585
SGOT(IU/L)	41(29-52)	42(28-52)	0.8133
SGPT(IU/L)	40(30-42)	40(27-45)	0.7127
ALP(IU/L)	225(175-263)	209(170-250)	0.4125
Total Protein(g/dl)	6.925(6.5-9.2)	6.85(6.5-7.61)	0.3224
Albumin(g/dl)	3.75(3.59-4)	3.75(3.52-3.95)	0.4972
Globulin(g/dl)	3.06(2.83-3.22)	3.05(2.8-3.21)	0.533
Gamma Glutamyl Transferase	32(25-51.38)	32(25-48)	0.9541
Renal Function Tests			
Urea(mg/dl)	34(26-41)	32(26-39)	0.7184
Creatinine(mg/dl)	1.17(0.99-1.38)	1.1(0.92-1.31)	0.0345
Uric acid(mg/dl)	5.365(4.82-6.17)	5.42(4.68-6.17)	0.9545
Minerals and Electrolytes			
Calcium(mg/dl)	8.46(8.2-8.7)	8.46(8.2-8.72)	0.5553
Phosphorous(mg/dl)	3.68(3.1-4.92)	3.7(3.2-4.5)	0.403
Sodium(mmol/L)	136.45(132.5-138.5)	136.5(134-139)	0.3101
Potassium(mmol/L)	3.975(3.74-4.22)	3.92(3.72-4.27)	0.8566

Table 2
Laboratory findings of patients admitted in second wave of COVID-19

	Female (n=189)	Male (n=394)	P value
	Median (IQR)	Median (IQR)	
Haematological Parameters			
Hemoglobin	12.2(11-13.4)	12.3(10.9-13.3)	0.7849
Platelet	2.22(1.54-2.72)	2.29(1.64-2.7)	0.1967
RBC	4.56(4.03-4.92)	4.495(4.04-4.92)	0.868
PCV	36.93(34.4-39.9)	36.93(33.8-40.6)	0.5823
MCV	84.35(81.1-88.7)	85(81.8-89.6)	0.2215
MCH	28.14(26.9-29.3)	28.14(26.6-29.7)	0.8379
MCHC	33.1(31.9-33.4)	32.8(31.8-33.3)	0.4353
Total Leukocyte Count	11730(7110-14260)	11625(7880-14680)	0.9063
Liver Function Tests			
Total Bilirubin	0.74(0.67-0.88)	0.75(0.68-0.88)	0.5391
Direct Bilirubin	0.23(0.22-0.29)	0.24(0.22-0.3)	0.7553
SGOT	56.34(36-58)	49(31-56.34)	0.1492
SGPT	55(36-55.6)	50(30-55.6)	0.169
ALP	241(196-256)	237(189-244)	0.3708
Total Protein	6.17(5.92-6.4)	6.25(5.95-6.4)	0.1127
Albumin	3.29(2.99-3.3)	3.3(3.01-3.41)	0.1351
Globulin	3.1(2.86-4.41)	3.07(2.81-3.91)	0.3939
Gamma Glutamyl	39(28-44)	38(28-44)	0.9125

Transferase			
Renal Function Tests			
Urea	51(37-53)	49(36-52)	0.4214
Creatinine	1.2(1.01-1.59)	1.2(1.03-1.59)	0.4804
Uric acid	4.92(4.25-6.2)	4.92(4.21-6.2)	0.4285
Minerals and Electrolytes			
Calcium	8.35(8.15-8.8)	8.4(8.16-8.9)	0.2694
Phosphorous	3.69(3.4-4.5)	3.57(3.4-4.15)	0.3308
Sodium	132.2(126.6-136.2)	133.6(126.6-136.4)	0.2412
Potassium	4.07(3.62-5.1)	4.08(3.59-5.1)	0.8683

Between varying grades of COVID-19 severity of the patients in the first wave, significant differences were observed in the parameters PCV ($p=0.004$) and Sodium ($p=0.011$) (Table 3). Similarly, in wave second in Total Leukocyte Count ($p<0.001$), Total Protein ($p=0.017$), and Globulin ($p<0.001$) (Table 4).

Table 3
Laboratory findings of patients admitted in first wave of COVID-19 based on severity of the disease

	Mild (n=222)	Moderate (n=56)	Severe (n=78)	P value
	Median (IQR)	Median (IQR)	Median (IQR)	
Haematological Parameters				
Hemoglobin	12.15(11.1-13.3)	11.6(10.55-13.3)	12.4(11.1-13.8)	0.107
Platelet	2.11(1.5-3)	2.155(1.48-2.82)	2.205(1.45-2.9)	0.956
RBC	4.28(3.77-4.76)	4.45(4.04-4.85)	4.435(3.9-5.22)	0.248
PCV	37.7(33.8-42.1)	37(32.7-40)	39.85(35.3-45.5)	0.004
MCV	86.9(81.5-92.4)	83.55(75.8-91)	85.4(80-89.7)	0.116
MCH	28.8(26.6-30.2)	28.05(28.85-31.05)	28.65(26.5-29.7)	0.920
MCHC	32.6(31.6-33.3)	32.85(31.95-33.4)	32.95(31.7-33.5)	0.290
Total Leukocyte Count	7280(5350-10290)	6305(4620-9470)	6310(4740-10720)	0.177
Liver Function Tests				
Total Bilirubin	0.72(0.67-0.86)	0.745(0.69-0.86)	0.71(0.68-0.85)	0.319
Direct Bilirubin	0.24(0.22-0.28)	0.23(0.22-0.25)	0.24(0.22-0.25)	0.252
SGOT	42.6(30-52)	37.5(26-54.5)	40.5(29-50)	0.600
SGPT	40(30-42)	37(24-52)	40(32-42)	0.484
ALP	215(179-254)	219.5(168.5-	196(132-242)	0.077

		270.5)		
Total Protein	6.85(6.45-7.71)	6.82(6.515-8.505)	6.925(6.59-7.8)	0.772
Albumin	3.75(3.6-4)	3.75(3.4-3.855)	3.75(3.41-3.89)	0.063
Globulin	3.05(2.79-3.2)	3.05(2.885-3.21)	3.1(2.9-3.22)	0.310
Gamma Glutamyl Transferase	33(25-49)	35.5(25-51.38)	30(21.2-41)	0.242
Renal Function Tests				
Urea	33(27-41)	34(28-39.5)	31(26-37)	0.242
Creatinine	1.1(0.95-1.31)	1.135(0.945-1.38)	1.11(0.95-1.32)	0.918
Uric acid	5.4(4.8-6)	5.42(4.34-6.025)	5.42(4.68-6.7)	0.185
Minerals and Electrolytes				
Calcium	8.52(8.2-8.72)	8.46(8.12-8.72)	8.34(8.19-8.6)	0.054
Phosphorous	3.68(3.12-4.22)	3.56(3.18-4.12)	3.88(3.4-4.92)	0.571
Sodium	136.8(134-139.2)	135.7(133.05-137.8)	134.85(128.3-138.4)	0.011
Potassium	3.91(3.7-4.23)	4.06(3.74-4.37)	4(3.74-4.22)	0.699

Table 4
Laboratory findings of patients admitted in second wave of COVID-19 based on severity of the disease

	Mild (n=401)	Moderate (n=62)	Severe (n=120)	P value
	Median (IQR)	Median (IQR)	Median (IQR)	
Haematological Parameters				
Hemoglobin	12.2 (10.8-13.1)	12.47(11.5-14.1)	12.435 (11.3-13.6)	0.461
Platelet	2.29 (1.64-2.74)	2.29 (1.87-2.67)	2.29(1.53-2.35)	0.965
RBC	4.47 (3.96-4.92)	4.6(4.23-4.92)	4.6 (4.165- 4.92)	0.344
PCV	36.93 (33.7-39.6)	37.12(34.8-43.3)	37.015 (34.4-40.85)	0.421
MCV	84.6 (81.6-89.1)	84.95(81.6-89.1)	84.425(81.65-89.8)	0.934
MCH	28.14 (26.6-29.5)	28.2(27.3-29.6)	28.14 (25.55-29.85)	0.410
MCHC	32.8 (31.8-33.4)	33.05(32-33.4)	32.9(32-33.4)	0.775
Total Leukocyte Count	10550 (7100-13680)	11788.31(8540-15120)	11900 (9320-17475)	<0.0001
Liver Function Tests				
Total Bilirubin	0.75 (0.68-0.88)	0.745(0.65-0.88)	0.74(0.68-0.85)	0.633
Direct Bilirubin	0.23 (0.22-0.86)	0.24(0.22-0.28)	0.23(0.215-0.26)	0.308
SGOT	54 (34-56.34))	52(34-59)	46.5(31-59)	0.270
SGPT	55 (32-55.6)	55.6(36-59)	45(32-58.5)	0.151

ALP	241 (195-247)	230.5(186-242)	220.5(182-250)	0.542
Total Protein	6.28 (5.96-6.4)	6.26(5.9-6.4)	6.12(5.895-6.4)	0.017
Albumin	3.3 (3.02-3.4)	3.195(2.9-3.3)	3.215(2.995-3.415)	0.142
Globulin	3.1 (2.83-4.41)	3.105(2.9-4.41)	2.965(2.78-3.205)	<0.0001
Gamma Glutamyl Transferase	39 (29-44)	42(25-48)	32(26-44)	0.051
Renal Function Tests				
Urea	52(37-52)	45.5(36-52)	48(35-58.5)	0.238
Creatinine	1.21(1.02-1.59)	1.17(1.03-1.59)	1.18(1.02-1.59)	0.529
Uric acid	4.92(4.21-6.2)	4.82(4.26-6.2)	4.88(4.21-5.9)	0.154
Minerals and Electrolytes				
Calcium	8.4(8.18-8.94)	8.37(8.2-8.85)	8.31 (8.12-8.7)	0.306
Phosphorous	3.65(3.4-5.4)	3.6(3.4-4.2)	3.5 (3.21-3.97)	0.067
Sodium	132.7(126.6-136.2)	132.7(126.6-137.6)	134.35(129.05-138.55)	0.062
Potassium	4.12(3.65-5.1)	4.08(3.56-5.1)	3.92(3.50-4.56)	0.160

Comparing the parameters between first and wave second in patients with mild severity, parameters including MCV ($p=0.024$), MCH ($p=0.031$), Total Leukocyte Count ($p<0.001$), SGOT ($p<0.001$), SGPT ($p<0.001$), APL ($p=0.003$), Total Protein ($p<0.001$), Albumin ($p<0.001$), Globulin ($p<0.001$), Gamma Glutamyl Transferase ($p=0.023$), Urea ($p<0.001$), Creatinine ($p<0.001$), Uric acid ($p=0.030$), Sodium ($p<0.001$) and Potassium($p<0.001$), showed significant differences. Similarly, in patients with moderate severity, parameters such as Hemoglobin ($p=0.015$), Total Leukocyte Count ($p<0.001$), SGOT ($p=0.011$), SGPT ($p=0.001$), Total Protein ($p<0.001$), Albumin ($p<0.001$), Globulin ($p=0.016$), Urea ($p<0.001$) and Sodium ($p<0.001$) perimeters were significantly different between first and second wave. In severe patients, PCV ($p=0.001$), Total Leukocyte Count ($p<0.001$), SGOT ($p=0.026$), SGPT ($p=0.023$), Total Protein ($p<0.001$), Albumin ($p<0.001$), Urea ($p<0.001$), Creatinine ($p<0.001$), Uric acid ($p<0.001$) and Phosphorous ($P=0.038$) showed significant difference between first and second wave (Table 5).

Table 5
Comparison of parameters between first and second wave in different grades of severity of COVID-19 disease

Parameters	Severity grade	Wave 1 (n=356)	Wave 2 (n=583)	P value
Haematological Parameters				
Hemoglobin	Mild	12.15(11.1-13.3)	12.2 (10.8-13.1)	0.519
	Moderate	11.6(10.55-13.3)	12.47(11.5-14.1)	0.015
	Severe	12.4(11.1-13.8)	12.435 (11.3-13.6)	0.745
Platelet	Mild	2.11(1.5-3)	2.29 (1.64-2.74)	0.844
	Moderate	2.155(1.48-	2.29 (1.87-2.67)	0.840

		2.82)		
	Severe	2.205(1.45-2.9)	2.29(1.53-2.35)	0.735
RBC	Mild	4.28(3.77-4.76)	4.47 (3.96-4.92)	0.090
	Moderate	4.45(4.04-4.85)	4.6(4.23-4.92)	0.224
	Severe	4.435(3.9-5.22)	4.6 (4.165-4.92)	0.546
PCV	Mild	37.7(33.8-42.1)	36.93 (33.7-39.6)	0.119
	Moderate	37(32.7-40)	37.12(34.8-43.3)	0.163
	Severe	39.85(35.3-45.5)	37.015 (34.4-40.85)	0.001
MCV	Mild	86.9(81.5-92.4)	84.6 (81.6-89.1)	0.024
	Moderate	83.55(75.8-91)	84.95(81.6-89.1)	0.498
	Severe	85.4(80-89.7)	84.425(81.65-89.8)	0.812
MCH	Mild	28.8(26.6-30.2)	28.14 (26.6-29.5)	0.031
	Moderate	28.05(28.85-31.05)	28.2(27.3-29.6)	0.905
	Severe	28.65(26.5-29.7)	28.14 (25.55-29.85)	0.273
MCHC	Mild	32.6(31.6-33.3)	32.8 (31.8-33.4)	0.114
	Moderate	32.85(31.95-33.4)	33.05(32-33.4)	0.662
	Severe	32.95(31.7-33.5)	32.9(32-33.4)	0.991
Total Leukocyte Count	Mild	7280(5350-10290)	10550 (7100-13680)	<0.0001
	Moderate	6305(4620-9470)	11788.31(8540-15120)	<0.0001
	Severe	6310(4740-10720)	11900 (9320-17475)	<0.0001
Liver Function Tests				
Total Bilirubin	Mild	0.72(0.67-0.86)	0.75 (0.68-0.88)	0.215
	Moderate	0.745(0.69-0.86)	0.745(0.65-0.88)	0.265
	Severe	0.71(0.68-0.85)	0.74(0.68-0.85)	0.911
Direct Bilirubin	Mild	0.24(0.22-0.28)	0.23 (0.22-0.86)	0.617
	Moderate	0.23(0.22-	0.24(0.22-0.28)	0.060

		0.25)		
	Severe	0.24(0.22-0.25)	0.23(0.215-0.26)	0.478
SGOT	Mild	42.6(30-52)	54 (34-56.34)	<0.0001
	Moderate	37.5(26-54.5)	52(34-59)	0.011
	Severe	40.5(29-50)	46.5(31-59)	0.026
SGPT	Mild	40(30-42)	55 (32-55.6)	<0.0001
	Moderate	37(24-52)	55.6(36-59)	0.001
	Severe	40(32-42)	45(32-58.5)	0.023
ALP	Mild	215(179-254)	241 (195-247)	0.003
	Moderate	219.5(168.5-270.5)	230.5(186-242)	0.705
	Severe	196(132-242)	220.5(182-250)	0.033
Total Protein	Mild	6.85(6.45-7.71)	6.28 (5.96-6.4)	<0.0001
	Moderate	6.82(6.515-8.505)	6.26(5.9-6.4)	<0.0001
	Severe	6.925(6.59-7.8)	6.12(5.895-6.4)	<0.0001
Albumin	Mild	3.75(3.6-4)	3.3 (3.02-3.4)	<0.0001
	Moderate	3.75(3.4-3.855)	3.195(2.9-3.3)	<0.0001
	Severe	3.75(3.41-3.89)	3.215(2.995-3.415)	<0.0001
Globulin	Mild	3.05(2.79-3.2)	3.1 (2.83-4.41)	<0.0001
	Moderate	3.05(2.885-3.21)	3.105(2.9-4.41)	0.016
	Severe	3.1(2.9-3.22)	2.965(2.78-3.205)	0.223
Gamma Glutamyl Transferase	Mild	33(25-49)	39 (29-44)	0.023
	Moderate	35.5(25-51.38)	42(25-48)	0.907
	Severe	30(21.2-41)	32(26-44)	0.264
Renal Function Tests				
Urea	Mild	33(27-41)	52(37-52)	<0.0001
	Moderate	34(28-39.5)	45.5(36-52)	<0.0001
	Severe	31(26-37)	48(35-58.5)	<0.0001
Creatinine	Mild	1.1(0.95-1.31)	1.21(1.02-1.59)	<0.0001
	Moderate	1.135(0.945-1.38)	1.17(1.03-1.59)	0.055
	Severe	1.11(0.95-1.32)	1.18(1.02-1.59)	0.091
Uric acid	Mild	5.4(4.8-6)	4.92(4.21-6.2)	0.030
	Moderate	5.42(4.34-6.025)	4.82(4.26-6.2)	0.476
	Severe	5.42(4.68-6.7)	4.88(4.21-5.9)	<0.0001
Minerals and Electrolytes				
Calcium	Mild	8.52(8.2-8.72)	8.4(8.18-8.94)	0.727
	Moderate	8.46(8.12-	8.37(8.2-8.85)	0.985

		8.72)		
	Severe	8.34(8.19-8.6)	8.31 (8.12-8.7)	0.910
Phosphorous	Mild	3.68(3.12-4.22)	3.65(3.4-5.4)	0.065
	Moderate	3.56(3.18-4.12)	3.6(3.4-4.2)	0.396
	Severe	3.88(3.4-4.92)	3.5 (3.21-3.97)	0.038
Sodium	Mild	136.8(134-139.2)	132.7(126.6-136.2)	<0.0001
	Moderate	135.7(133.05-137.8)	132.7(126.6-137.6)	.013
	Severe	134.85(128.3-138.4)	134.35(129.05-138.55)	0.418
Potassium	Mild	3.91(3.7-4.23)	4.12(3.65-5.1)	0.000
	Moderate	4.06(3.74-4.37)	4.08(3.56-5.1)	0.378
	Severe	4(3.74-4.22)	3.92(3.50-4.56)	0.941

We performed ROC analysis to assess the distinguishing capability of various parameters for predicting the severity of the disease. Based on ROC analysis, total leukocyte count was observed to have an increased ability to distinguish between mild and severe COVID-19 in the second wave when compared to the first wave. We considered those parameters that have an area under the curve of more than 0.6. Based on ROC analysis, PCV and total leukocyte count were observed to have an increased ability to distinguish between mild and severe COVID-19 in the first and second wave respectively (Table 6, Figure1 and Figure 2).

Table 6
ROC analysis of various parameters in assessing the ability to distinguish between mild and severe disease in first and second wave of COVID-19

	Wave 1 (n=301)			Wave 2 (n=521)		
	Area	Std. Err.	[95% Conf. Interval]	Area	Std. Err.	[95% Conf. Interval]
Haematological Parameters						
Hemoglobin	0.5454	0.0379	0.47109 0.61963	0.5528	0.0295	0.49487 0.61068
Platelet	0.4912	0.0388	0.41512 0.56721	0.4791	0.0304	0.41952 0.53861
RBC	0.5434	0.04	0.46496 0.62184	0.5434	0.0284	0.48764 0.59908
PCV	0.61	0.0386	0.53432 0.68559	0.5424	0.0303	0.48298 0.60181
MCV	0.4411	0.0366	0.36942 0.51271	0.503	0.0305	0.44330 0.56274
MCH	0.4881	0.0386	0.41237 0.56384	0.4931	0.0309	0.43256 0.55374
MCHC	0.549	0.04	0.47071 0.62735	0.5204	0.0294	0.46278 0.57804

Total Leukocyte Count	0.4379	0.0404	0.35885 0.51705	0.6257	0.0296	0.56762 0.68373
Liver Function Tests						
Total Bilirubin	0.5134	0.0374	0.44006 0.58678	0.4821	0.0302	0.42280 0.54134
Direct Bilirubin	0.4824	0.037	0.40999 0.55488	0.4507	0.0287	0.39443 0.50692
SGOT	0.4661	0.0377	0.39219 0.54002	0.4844	0.0312	0.42324 0.54551
SGPT	0.5067	0.0377	0.43282 0.58063	0.4867	0.0308	0.42642 0.54704
ALP	0.4148	0.0419	0.33271 0.49688	0.4549	0.0307	0.39471 0.51506
Total Protein	0.5274	0.0369	0.45506 0.59973	0.4366	0.0298	0.37815 0.49496
Albumin	0.4479	0.038	0.37343 0.52234	0.4739	0.031	0.41318 0.53453
Globulin	0.5572	0.0386	0.48162 0.63273	0.4109	0.028	0.35607 0.46565
Gamma Glutamyl Transferase	0.448	0.0386	0.37237 0.52369	0.4443	0.0304	0.38467 0.50386
Renal Function Tests						
Urea	0.4369	0.0385	0.36141 0.51240	0.5016	0.0297	0.44331 0.55991
Creatinine	0.517	0.0376	0.44332 0.59066	0.4674	0.029	0.41052 0.52421
Uric acid	0.5595	0.0405	0.48010 0.63896	0.4563	0.0287	0.40010 0.51246
Minerals and Electrolytes						
Calcium	0.4081	0.0367	0.33605 0.48010	0.4417	0.0286	0.38556 0.49776
Phosphorous	0.5338	0.0374	0.46048 0.60719	0.4077	0.0288	0.35119 0.46414
Sodium	0.3926	0.0389	0.31637 0.46890	0.5612	0.0298	0.50266 0.61966
Potassium	0.5083	0.0386	0.43261 0.58406	0.4081	0.0291	0.35119 0.46508

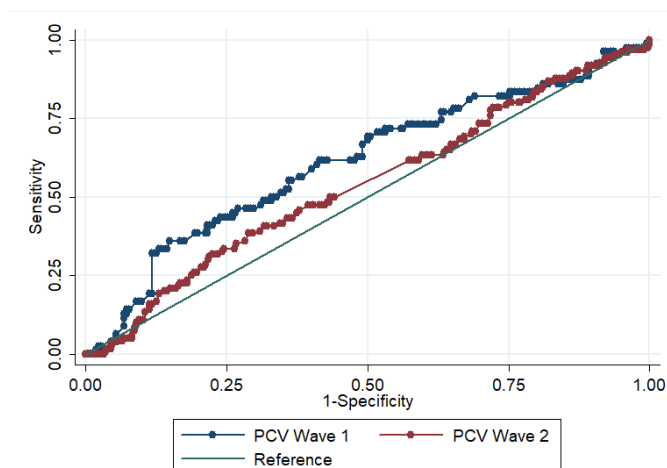


Figure 1. ROC Curve of PCV between Mild and Severe COVID-19 in first and second wave of COVID-19

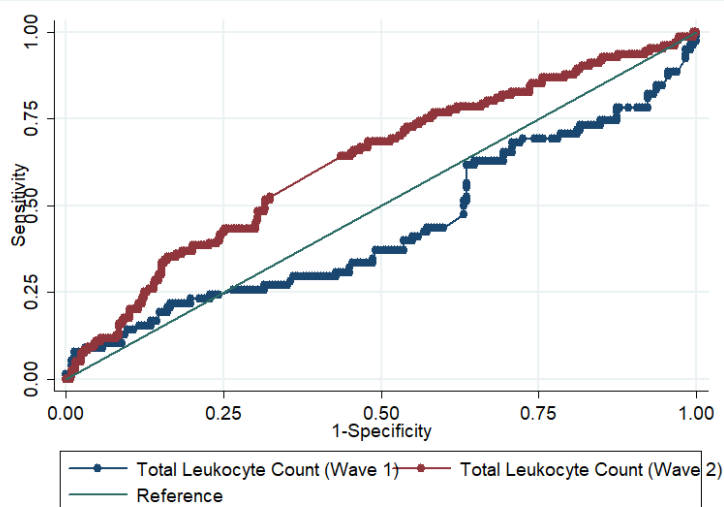


Figure 2. ROC Curve of TLC between Mild and Severe COVID-19 in first and second wave of COVID-19

Similarly, we observed hemoglobin and PCV to have an increased ability to distinguish between moderate and severe disease in the first COVID-19 wave. No parameters were seen to satisfactorily distinguish between moderate and severe disease in the second COVID-19 (Table 7, Figure 3 and Figure 4).

Table 7
ROC analysis of various parameters in assessing the ability to distinguish
between moderate and severe disease in first and second wave of COVID-19

	Wave 1 (n=135)			Wave 2(n=182)		
	Area	Std. Err.	[95% Conf. Interval]	Area	Std. Err.	[95% Conf. Interval]
Haematological Parameters						
Hemoglobin	0.6057	0.0491	0.50950 0.70192	0.4587	0.047	0.36653 0.55079
Platelet	0.5026	0.0504	0.40374 0.60144	0.4734	0.0449	0.38528 0.56145
RBC	0.4924	0.0501	0.39412 0.59059	0.4619	0.0466	0.37051 0.55332
PCV	0.6552	0.0469	0.56318 0.74721	0.4566	0.0469	0.36465 0.54847
MCV	0.5139	0.0523	0.41146 0.61643	0.4957	0.0457	0.40611 0.58535
MCH	0.5083	0.0515	0.40745 0.60920	0.4566	0.0449	0.36856 0.54470
MCHC	0.5112	0.0501	0.41299 0.60950	0.4877	0.0459	0.39770 0.57763
Total Leukocyte Count	0.4909	0.051	0.39086 0.59092	0.5579	0.0447	0.47036 0.64539
Liver Function Tests						
Total Bilirubin	0.4487	0.0505	0.34976 0.54768	0.5055	0.0463	0.41480 0.59622
Direct Bilirubin	0.5495	0.0495	0.45250 0.64646	0.4274	0.0447	0.33988 0.51496
SGOT	0.5127	0.0536	0.40764 0.61777	0.4665	0.044	0.38020 0.55273
SGPT	0.5525	0.0522	0.45023 0.65480	0.455	0.0443	0.36812 0.54183
ALP	0.419	0.0493	0.32234 0.51571	0.4796	0.0444	0.39263 0.56651
Total Protein	0.5262	0.0521	0.42415 0.62826	0.4591	0.0466	0.36770 0.55045
Albumin	0.5376	0.0503	0.43888 0.63624	0.5547	0.0437	0.46896 0.64044
Globulin	0.5358	0.0504	0.43706 0.63446	0.3694	0.0445	0.28208 0.45663
Gamma Glutamyl Transferase	0.4296	0.0522	0.32729 0.53190	0.4555	0.0473	0.36280 0.54822
Renal Function Tests						
Urea	0.4452	0.0507	0.34593 0.54454	0.5245	0.0442	0.43786 0.61120

Creatinine	0.5103	0.0513	0.40973 0.61096	0.498	0.0455	0.40889 0.58708
Uric acid	0.5821	0.0501	0.48396 0.68023	0.4821	0.0463	0.39141 0.57283
Minerals and Electrolytes						
Calcium	0.4128	0.0509	0.31318 0.51251	0.445	0.0455	0.35581 0.53411
Phosphorous	0.5494	0.0509	0.44969 0.64905	0.4142	0.0443	0.32734 0.50116
Sodium	0.4414	0.0494	0.34464 0.53817	0.5388	0.0458	0.44894 0.62862
Potassium	0.4712	0.0509	0.37149 0.57093	0.4377	0.0466	0.34642 0.52899

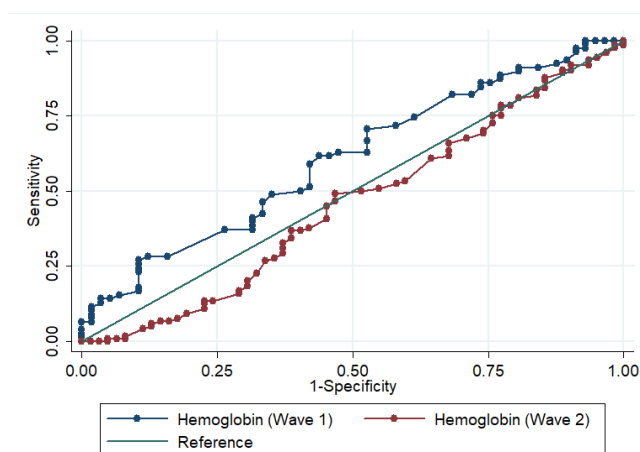


Figure 3. ROC Curve of Hb between Moderate and Severe COVID-19 in first and second wave of COVID-19

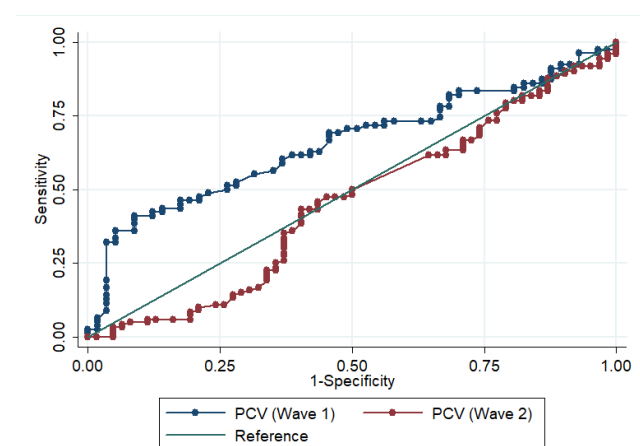


Figure 4. ROC Curve of PCV between Moderate and Severe COVID-19 in first and second wave of COVID-19

However, between moderate and severe COVID-19 disease, we were not able to identify any parameter with satisfactorily distinguishing capabilities (Table 8).

Table 8
ROC analysis of various parameters in assessing the ability to distinguish between mild and moderate disease in first and second wave of COVID-19

	Wave 1 (n=279)			Wave 2 (n=463)		
	Area	Std. Err.	[95% Conf. Interval]	Area	Std. Err.	[95% Conf. Interval]
Haematological Parameters						
Hemoglobin	0.4344	0.0427	0.35073 0.51816	0.583	0.0404	0.50381 0.66227
Platelet	0.4824	0.043	0.39816 0.56669	0.5068	0.0386	0.43102 0.58250
RBC	0.5557	0.0423	0.47277 0.63871	0.5771	0.0382	0.50225 0.65188
PCV	0.4616	0.0408	0.38165 0.54153	0.5731	0.0408	0.49317 0.65303
MCV	0.4287	0.0439	0.34255 0.51483	0.508	0.0401	0.42936 0.58669
MCH	0.4849	0.0458	0.39519 0.57457	0.5353	0.039	0.45892 0.61167
MCHC	0.5478	0.0429	0.46365 0.63199	0.5323	0.0389	0.45597 0.60862
Total Leukocyte Count	0.4352	0.0465	0.34411 0.52622	0.5782	0.0378	0.50412 0.65222
Liver Function Tests						
Total Bilirubin	0.5592	0.0428	0.47535 0.64300	0.473	0.0412	0.39218 0.55384
Direct Bilirubin	0.4381	0.0389	0.36176 0.51444	0.5223	0.0374	0.44904 0.59548
SGOT	0.4622	0.0483	0.36760 0.55681	0.5176	0.0389	0.44136 0.59387
SGPT	0.4523	0.0471	0.35995 0.54468	0.5361	0.0386	0.46048 0.61168
ALP	0.4957	0.0471	0.40338 0.58805	0.4696	0.0384	0.39434 0.54493
Total Protein	0.4993	0.043	0.41504 0.58352	0.4832	0.0413	0.40237 0.56413
Albumin	0.4182	0.0445	0.33099 0.50533	0.4164	0.0375	0.34290 0.48998
Globulin	0.5162	0.0438	0.43035 0.60209	0.5304	0.0375	0.45688 0.60389
Gamma Glutamyl Transferase	0.5365	0.0475	0.44330 0.62965	0.5033	0.0436	0.41788 0.58875

Renal Function Tests						
Urea	0.493	0.043	0.40866 0.57732	0.4739	0.0368	0.40180 0.54604
Creatinine	0.4988	0.0429	0.41464 0.58296	0.467	0.0377	0.39321 0.54083
Uric acid	0.4781	0.0464	0.38723 0.56897	0.4787	0.0393	0.40160 0.55581
Minerals and Electrolytes						
Calcium	0.4903	0.044	0.40399 0.57663	0.4915	0.0383	0.41646 0.56644
Phosphorous	0.4859	0.0415	0.40452 0.56729	0.4887	0.0386	0.41311 0.56425
Sodium	0.4176	0.0404	0.33836 0.49676	0.5249	0.0405	0.44557 0.60422
Potassium	0.5365	0.0451	0.44808 0.62487	0.4779	0.0423	0.39512 0.56076

Discussion

A dual-wave pattern has been reported in most countries during the COVID-19 pandemic, with the first wave occurring during spring followed by a second wave in late summer and autumn. Empirical data showed that the characteristics of the pandemic varied between the two periods [9]. One of the major reasons for the varying manifestation of COVID-19 disease in the first and second wave have been attributed to the emergence of the newer variant of SARS-CoV-2 in early summer 2020 in Spain [10]. The most frequent signs and symptoms in both waves were fever, dyspnoea, pneumonia, and cough. The frequently associated co morbidities were cardiovascular diseases, type 2 diabetes mellitus, and chronic neurological diseases. Patients from the second wave were observed to have more frequent presentations of renal and gastrointestinal symptoms, were more often treated with non-invasive mechanical ventilation and corticosteroids. In addition, several differences in mortality risk factors were also observed [9]. Although the predominant symptoms of infection (fever, dyspnoea, pneumonia cough) were similar in both waves, the patients in the second wave also presented often with renal (acute kidney failure) and gastrointestinal symptoms (vomiting, abdominal pain)[11]. Multiple studies have documented the pattern of differences between the first and second waves of COVID-19 diseases. Asia and Europe [9, 12-14] experienced a second wave several weeks to several months ahead of the US but few studies to date have characterized resurgences with respect to clinical variables in detail. Japan had a second wave that affected the younger population with fewer co morbidities, fewer severe patients at admission, and reduced mortality [12]. Similarly, Spain and Italy also had their second wave affecting the younger population, needed less-invasive mechanical ventilation, and had lower mortality [9,13].

The differences in epidemiological characteristics and variation in strains lead to, apart from differences in clinical presentation, varying patterns of biochemical and haematological parameters in both waves of COVID-19. Various studies have also assessed the differences in biochemical and haematological parameters between the first and second waves of COVID-19. Mollinedo-Gajate et al have

observed that Interleukin-6 and d-dimer levels were higher in the first wave of COVID-19 than in the second wave [15]. Asghar et al, in their study among the Pakistani population, have observed higher neutrophils and lymphocytes in the second wave of COVID-19 when compared with the first wave. They also demonstrated lymphocytes to be able to predict the severity of the second wave of COVID-19, which was attributed to the dampening of inflammatory response from early use of immune-suppressants [16].

In our study, we observed a varying pattern of biochemical and hematological parameters between the first and second waves of COVID-19. While PCV and sodium were observed to be significantly different between the different grades of severity of COVID-19 in the first wave, the second wave had Total Leukocyte Count, Total Protein, and Globulin. When we compared the similar grades of severity of COVID-19 between the first and second wave, we observed that Total Leukocyte Count, SGOT, SGPT, Total Protein, Albumin, Globulin, and Urea to be significantly different in mild, moderate, and severe COVID-19. Our findings are in concordance with the study from our neighbouring country, where they also observed alteration in Leukocyte Count as a predominant feature of the second wave of COVID-19 [16]. Our ROC analysis demonstrated an increase in the distinguishing capabilities of Total Leukocyte Count between mild and Severe disease in the second wave of COVID-19 which was conspicuously absent in the first wave of COVID-19. Collectively, our study reported increased liver function deterioration and increased Total Leukocyte Count in patients from the Northern part of India presenting with COVID-19 during the second wave when compared with the first wave. The alteration in the pattern may be attributed to the aggressive treatment strategies adopted during the second wave of COVID-19 including the early use of immunosuppressants, anticoagulants, and excessive self-medication of antipyretics.

Conclusion

Our study highlights a significant difference in the pattern of biochemical and hematological parameters between the first and second waves of COVID-19. When compared with the first wave of COVID-19 in northern parts of India, the second wave was characterized by increased liver function deterioration and increased Total Leukocyte Count. Further studies are required to assess the aetiology behind the observed variation in biochemical and haematological parameters between the first and second waves of COVID-19.

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