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Research Article

ROLE OF BIOCHEMICAL MARKERS AMONG COVID-19 PATIENTS AND THEIR ASSOCIATION WITH SEVERITY OF DISEASE

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ABSTRACT

Objectives: The present study was aimed to evaluate the levels of biomarkers (D-dimer, Procalcitonin [PCT], lactate dehydrogenase [LDH], interleukin [IL]-6, and C-reactive protein [CRP]) among coronavirus disease 2019 (COVID-19) patients visiting/admitted in Government Medical College Jammu and analyze their association with the severity of disease.

Methods: The study was conducted on 100 COVID-19-positive patients 18–75 years of age of either sex, visiting/admitted in Government Medical College Jammu, for a period of 6 months. The serum glucose levels, creatine phosphokinase, LDH, liver function tests, renal function tests, and various biochemical COVID-19 markers such as CRP, serum ferritin, IL-6, D-dimer, and PCT were analyzed.

Results: In the present study, it was observed that when patients were divided according to the severity of disease, there was a significant difference in the mean levels of total bilirubin, serum sodium, serum potassium, D-dimer, SpO_2, and duration of symptoms in a mild and severe group of patients, while there was no significant difference in the parameters such as IL-6, ferritin, CRP, PCT, LDH, and other routine biochemistry parameters. When a comparison was done between both the groups according to SpO_2, only the mean levels of serum total bilirubin were significant.

Conclusion: Thus, it is concluded that serum bilirubin was significantly elevated in mild, moderate, and severe groups of patients. Further research should be conducted to find some biomarkers which are specific to COVID-19 only.

Keywords: COVID-19, biochemical markers, pandemic, severity

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INTRODUCTION

The infectious coronavirus disease 2019 (COVID-19) came to light in late December 2019, particularly in Wuhan China. SARS-CoV-2 of *Coronaviridae* family of virus is the causative agent of this disease [1]. With the passage of time, the disease that started from Wuhan, China spread worldwide affecting about 200 countries, thus causing a pandemic. The declaration of COVID-19, a global pandemic by the WHO came on 11th of March 2020 [2]. The respiratory system is the chief target of this highly contagious virus. It spreads by aerosols which can stick and contaminate any surface as well as remain viable for several days [3,4]. The symptoms of COVID-19 can be mild-to-severe. It can either show similarity with mild flu-like symptoms resulting in cough, shortness of breath, or fatigue or it can also cause serious complications such as breathing difficulties, pneumonia as well as heart and kidney failure, particularly in high-risk patients such as old people and those with comorbidities such as hypertension, diabetes, and heart problems resulting in death. As of May 13, 2020, a total of 4,170,424 confirmed cases of COVID-19 (with 287,399 deaths) have been reported in more than 210 affected countries worldwide (WHO Situation Report 114) [5].

The methods for the prognosis of severity of disease in COVID-19 patients are limited. However, previous evidences indicate that dimerized plasmin fragment (D-dimer), C reactive protein (CRP), lactate dehydrogenase (LDH), and procalcitonin (PCT), interleukin (IL)-6 play an important role in predicting severity associated with COVID-19 [6-8].

In the present situation, there is an urgent need for the identification of clinical and laboratory predictors of progression to serious and lethal

Table 1: Distribution of patients according to severity of disease

Severity of disease				
Disease severity	**Frequency**	**Percent**	**Valid percent**	**Cumulative percent**
Valid				
Mild	63	63.0	63.0	63.0
Moderate	23	23.0	23.0	86.0
Severe	14	14.0	14.0	100.0
Total	100	100.0	100.0	

Table 2: Distribution of patients according to gender and severity of disease

Sex * severity of disease cross-tabulation				
Gender-wise distribution	**Severity of disease**			**Total**
	Mild	**Moderate**	**Severe**	
Sex				
m				
Count	41	12	10	63
% within severity of disease	65.1	52.2	71.4	63.0
F				
Count	22	11	4	37
% within severity of disease	34.9	47.8	28.6	37.0
Total				
Count	63	23	14	100
% within severity of disease	100.0	100.0	100.0	100.0

Table 3: Descriptive statistics

Severity of disease	n	Mean	Standard Deviation	Minimum	Maximum	Percentiles		
						25th	50th (Median)	75th
Mild								
Blood sugar	63	204.386	99.6995	77.0	509.0	133.000	172.000	251.000
T. bilirubin	63	0.7179	0.31984	0.30	2.40	0.5000	0.7000	0.8000
T. protein	63	6.756	0.6703	4.9	8.0	6.300	6.800	7.200
ALK phos	63	110.524	42.5327	45.0	286.0	85.000	105.000	123.000
SGOT	63	76.876	117.8379	18.0	884.0	38.000	50.000	72.000
SGPT	63	113.263	166.3997	21.6	1025.0	44.000	67.000	90.000
S. urea	63	44.556	24.2957	17.0	136.0	29.000	37.000	49.000
S. creatinine	63	1.0989	0.71809	0.60	5.10	0.8000	0.9000	1.2000
NA	63	135.937	4.2155	126.0	147.0	133.000	136.000	139.000
K	63	4.1708	0.48715	3.00	5.50	4.0000	4.2000	4.5000
D-dimer	63	408.603	311.3979	45.0	1330.0	200.000	320.000	550.000
Procal	63	0.6452	3.48994	0.03	27.80	0.0600	0.1000	0.1800
IL-6	63	12.8225	21.63171	0.06	141.00	1.5000	3.8000	14.4000
S. ferritin	63	695.062	1262.8339	3.5	7214.0	171.100	332.000	773.000
CRP	63	31.9251	29.69614	4.90	171.00	18.6000	24.0000	36.4000
LDH	63	486.000	908.1654	52.0	5372.0	223.000	320.000	407.000
CPK	63	169.270	238.1446	20.0	1257.0	67.000	86.000	136.000
SpO_2	63	93.937	3.3451	84.0	99.0	92.000	94.000	96.000
Duration of Symptoms (days)	63	5.52	3.335	1	16	3.00	5.00	7.00
Severity of disease	63	1.00	0.000	1	1	1.00	1.00	1.00
Moderate								
Blood Sugar	23	264.696	153.7470	127.0	672.0	171.000	206.000	307.000
T. bilirubin	23	0.8435	0.40320	0.40	2.40	0.6000	0.8000	0.9000
T. protein	23	6.570	1.5218	4.0	9.3	6.000	6.800	7.700
ALK phos	23	109.870	62.0303	32.0	286.0	72.000	88.000	122.000
SGOT	23	97.304	174.0685	21.0	884.0	44.000	59.000	89.000
SGPT	23	117.348	205.2027	27.0	1025.0	42.000	63.000	86.000
S. urea	23	55.261	29.2656	20.0	130.0	24.000	52.000	77.000
S. creatinine	23	1.2478	0.78617	0.60	3.90	0.8000	0.9000	1.2000
NA	23	138.000	3.0600	131.0	143.0	135.000	139.000	140.000
K	23	4.3391	0.45301	3.30	5.10	4.0000	4.4000	4.5000
D-dimer	23	472.391	336.3066	113.0	1320.0	213.000	300.000	732.000
Procal	23	0.2161	0.27991	0.05	1.20	0.0700	0.1000	0.2800
IL-6	23	15.2826	14.32428	1.10	52.70	3.5000	12.0000	21.8000
S. ferritin	23	496.343	416.4505	92.8	1600.0	170.700	334.000	850.000
CRP	23	26.9522	12.89457	10.60	52.10	17.8000	23.6000	40.6000
LDH	23	394.783	180.4711	154.0	754.0	243.000	332.000	496.000
CPK	23	190.652	297.0552	39.0	1289.0	64.000	83.000	145.000
SpO_2	23	83.217	4.6118	76.0	94.0	80.000	83.000	88.000
Duration of Symptoms (days)	23	6.87	4.224	1	16	4.00	6.00	10.00
Severity of disease	23	2.00	0.000	2	2	2.00	2.00	2.00
Severe								
Blood sugar	14	212.071	110.6119	95.0	504.0	126.500	192.000	301.000
T. bilirubin	14	0.8143	0.12924	0.60	1.20	0.8000	0.8000	0.8000
T. protein	14	6.293	1.0594	4.0	8.9	5.750	6.400	6.550
ALK phos	14	118.643	46.9642	65.0	223.0	89.000	98.000	133.500
SGOT	14	61.429	33.4749	20.0	148.0	43.000	46.000	83.250
SGPT	14	83.357	56.1901	45.0	185.0	47.000	52.000	125.500
S. urea	14	60.857	29.5006	20.0	123.0	32.000	59.500	85.000
S. creatinine	14	1.1429	0.34130	0.70	1.70	0.9000	1.0000	1.5000
NA	14	139.143	8.4658	128.0	155.0	132.500	139.000	141.750
K	14	7.2286	9.73127	4.10	41.00	4.3000	4.6500	5.0000
D-dimer	14	1011.714	1147.3558	160.0	4660.0	382.750	600.500	1163.500
Procal	14	0.2250	0.30074	0.01	0.92	0.0675	0.0950	0.2525
IL-6	14	16.4214	21.80830	0.50	58.10	1.5500	5.9000	25.4750
S. ferritin	14	485.543	428.7867	86.2	1650.0	174.000	391.700	619.000
CRP	14	94.2929	132.53048	7.40	392.00	12.6000	21.6000	108.0000
LDH	14	356.286	126.8054	142.0	561.0	292.750	339.000	458.000
CPK	14	112.214	125.0318	37.0	534.0	63.500	81.000	88.250
SpO_2	14	67.929	6.9776	54.0	78.0	64.000	67.000	73.000
Duration of symptoms (days)	14	10.86	4.721	2	18	7.00	11.00	14.50
Severity of disease	14	3.00	0.000	3	3	3.00	3.00	3.00

SGOT: Serum glutamic-oxaloacetic transaminase, SGPT: Serum glutamic-pyruvic transaminase, IL: Interleukin, CRP: C-reactive protein, LDH: Lactate dehydrogenase, CPK: Creatine phosphokinase

forms. The prediction would allow clinicians to identify the patients at increased risk of going to a serious condition and will also help to formulate an effective strategy to fight against COVID-19 disease. However, until date no relevant information pertaining to the levels of these biomarkers and the severity of disease among COVID-19 patients visiting/admitted in Government Medical College, Jammu has been

reported so far. The present clinical practice suggests, in addition to the routine laboratory tests, determination of IL-6, D dimer, LDH, and transaminases, helps in identifying patients at risk of developing fatal complications and those who can be benefitted from anti-IL6 immunotherapies with drugs such as tocilizumab [9]. On the other hand, as most of clinical laboratories do not perform cytokine analysis routinely, some surrogate markers of infection such as ferritin and CRP associated to IL-6 have prognostic importance.

Hence, blood investigations play a primary role in the assessment of progression of disease. Some blood parameters are related with poor outcome of disease, such as serum ferritin, creatine kinase (CK) total, CK-MB, IL-6, PCT, and blood D-dimer levels [10].

Some biochemical markers are linked with the severity of coronavirus disease. Ferritin is one of the acute-phase reactants produced in various inflammatory conditions of the body. It not only restricts the availability of iron to the pathogens but also involved in the regulation of synthesis and release of cytokines which is ultimately responsible for the cytokine storm [11].

CRP is another acute-phase reactant, synthesized by the liver in various inflammatory conditions or infection. It also participates in the pro-inflammatory cycle by causing activation of inflammatory cytokines in the body [12].

PCT produced by the C-cells of the parathyroid gland, is also considered as a useful marker of severe systemic inflammation. The proinflammatory cytokines, such as tumor necrosis factor-alpha (TNF-a) and IL-6 mediates the release of PCT [13]. It had been found in various studies that PCT levels were lesser in seriously ill patients with viral infections and were much elevated in bacterial infections [14].

The most important immunopathological finding in COVID-19 is the cytokine storm [12]. The severity of COVID-19 depends on various proinflammatory cytokines that can ultimately contribute to the development of acute respiratory distress syndrome and multiple organ failure [15]. Keeping in view the above facts the present study was designed to evaluate the levels of biomarkers (D-dimer, PCT, LDH, IL-6, and CRP) among COVID-19 patients visiting admitted in Government Medical College Jammu and analyze their association with the severity of disease.

Table 4: Comparison of different parameters according to severity of disease

Test statistics[a,b]			
Parameters	**Chi-square**	**df**	**Asymp. Sig.**
Blood sugar	4.018	2	0.134
T. bilirubin	7.285	2	0.026*
T. protein	4.432	2	0.109
ALK phos	1.505	2	0.471
SGOT	0.939	2	0.625
SGPT	0.033	2	0.984
S. urea	5.780	2	0.056
S. creatinine	1.724	2	0.422
Na	6.636	2	0.036*
K	10.329	2	0.006**
D-dimer	7.614	2	0.022*
Procal	0.307	2	0.858
IL-6	3.418	2	0.181
S. ferritin	0.002	2	0.999
CRP	0.282	2	0.868
LDH	2.371	2	0.306
CPK	0.571	2	0.752
SpO2	67.290	2	<0.001**
Duration of Symptoms (days)	14.188	2	0.001**

[a]Kruskal–Wallis Test, [b]Grouping Variable: severity of disease, SGOT: Serum glutamic-oxaloacetic transaminase, SGPT: Serum glutamic-pyruvic transaminase, IL: Interleukin, CRP: C-reactive protein, LDH: Lactate dehydrogenase, CPK: Creatine phosphokinase. Total bilirubin- T.bilirubin, Total protein-T. protein

METHODS

A total of 100 patients including males and females suffering from COVID-19 of 18–75 years of age were enrolled for the study. The study was conducted in the Department of Biochemistry in collaboration with Department of Medicine, Government Medical College, Jammu for a period of 6 months, that is, from October 2021 to March 2022. The study was carried out after obtaining due approval from the Institutional Ethics Committee (Reference no. IEC/GMC/2021/642 dated: November 02, 2021) of the College. 5 mL of blood samples were withdrawn from the antecubital vein under aseptic conditions from each individual with his/her consent, duly following the guidelines and norms of the hospital. Blood samples were collected in plain, sodium fluoride, and sodium citrate vacutainers. The serum glucose levels, creatine phosphokinase, LDH, liver function tests, renal function tests, and various biochemical COVID-19 markers such as CRP, serum ferritin, IL-6, D-dimer, and PCT were analyzed. Blood glucose levels were estimated by Abbott Architect c-Systems by hexokinase method. The

Table 5: Comparison of various parameters in mild and moderate patients using Mann-whitney test

Parameters	**Mann–Whitney U**	**Wilcoxon W**	**Z**	**Asymp. Sig. (2-tailed)**
Blood sugar	524.000	2540.000	−1.957	0.050
T. bilirubin	537.000	2553.000	−1.849	0.064
T. protein	714.500	2730.500	−0.098	0.922
ALK phos	623.500	899.500	−0.986	0.324
SGOT	630.500	2646.500	−0.917	0.359
SGPT	711.500	987.500	−0.127	0.899
S. urea	561.500	2577.500	−1.591	0.112
S. creatinine	663.500	2679.500	−0.602	0.547
Na	486.500	2502.500	−2.331	0.020*
K	566.000	2582.000	−1.552	0.121
D-dimer	635.000	2651.000	−0.873	0.382
Procal	696.000	2712.000	−0.279	0.780
IL-6	536.500	2552.500	−1.835	0.066
S. ferritin	717.500	2733.500	−0.068	0.946
CRP	704.000	980.000	−0.200	0.841
LDH	584.500	2600.500	−1.366	0.172
CPK	712.000	988.000	−0.122	0.903
SpO_2	57.500	333.500	−6.540	<0.001**
Duration of symptoms (days)	596.500	2612.500	−1.256	0.209

[a]Grouping variable: Severity of disease, SGOT: Serum glutamic-oxaloacetic transaminase, SGPT: Serum glutamic-pyruvic transaminase, IL: Interleukin, CRP: C-reactive protein, LDH: Lactate dehydrogenase, CPK: Creatine phosphokinase. Na-Sodium , K-Potassium and p value < 0.05 is statistically significant.

Table 6: Comparison of various parameters in moderate and severe patients using Mann–Whitney test

Parameters	Mann–Whitney U	Wilcoxon W	Z	Asymp. Sig. (2-tailed)	Exact Sig. (2* [1-tailed Sig.])
Blood sugar	117.000	222.000	−1.379	0.168	0.175[b]
T. bilirubin	151.500	427.500	−0.317	0.751	0.769[b]
T. protein	120.000	225.000	−1.287	0.198	0.208[b]
ALK phos	123.000	399.000	−1.191	0.234	0.244[b]
SGOT	137.500	242.500	−0.737	0.461	0.467[b]
SGPT	154.000	259.000	−0.219	0.826	0.841[b]
S. urea	137.000	413.000	−0.753	0.451	0.467[b]
S. creatinine	144.000	420.000	−0.539	0.590	0.610[b]
Na	152.000	428.000	−0.284	0.776	0.793[b]
K	111.000	387.000	−1.572	0.116	0.122[b]
D-dimer	101.000	377.000	−1.880	0.060	0.062[b]
Procal	143.500	248.500	−0.551	0.581	0.588[b]
IL-6	134.000	239.000	−0.846	0.398	0.411[b]
S. ferritin	159.500	435.500	−0.047	0.963	0.963[b]
CRP	143.000	419.000	−0.564	0.573	0.588[b]
LDH	157.000	262.000	−0.125	0.900	0.914[b]
CPK	143.500	248.500	−0.549	0.583	0.588[b]
SpO_2	5.500	110.500	−4.884	<0.001**	0.000[b]
Duration of Symptoms (days)	85.500	361.500	−2.373	0.018*	0.017[b]

[a]Grouping Variable: severity of disease, bNot corrected for ties, SGOT: Serum glutamic-oxaloacetic transaminase, SGPT: Serum glutamic-pyruvic transaminase, IL: Interleukin, CRP: C-reactive protein, LDH: Lactate dehydrogenase, CPK: Creatine phosphokinase. SpO_2- oxygen saturation

Table 7: Mean age of patients in different SpO_2 groups

Group statistics					
	SpO_2 groups	n	Mean	Standard deviation	Standard error mean
Mean Age	<94	58	63.379	15.7300	2.0654
	>=94	41	60.634	14.2034	2.2182

Table 8: Gender-wise distribution of patients in different SpO_2 groups

Crosstab			
Gender	SpO_2 groups		Total
	<94	≥94	
Sex			
m			
Count	33	30	63
% within sex	52.4	47.6	100.0
F			
Count	25	12	37
% within sex	67.6	32.4	100.0
Total			
Count	58	42	100
% within sex	58.0	42.0	100.0

Table 9: Distribution of patients in different SpO_2 groups according to severity of disease

Crosstab			
Disease severity	SpO_2 groups		Total
	<94	≥94	
Severity of disease			
Mild			
Count	22	41	63
% within severity of disease	34.9	65.1	100.0
Moderate			
Count	22	1	23
% within severity of disease	95.7	4.3	100.0
Severe			
Count	14	0	14
% within severity of disease	100.0	0.0	100.0
Total			
Count	58	42	100
% within severity of disease	58.0	42.0	100.0

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-square	37.299[a]	2	<0.001**
Likelihood ratio	46.315	2	0.000
Linear-by-linear association	31.980	1	0.000
n of valid cases	100		

[a]0 cells (0.0%) have expected count <5. The minimum expected count is 5.88

ferritin levels were estimated in Abbott Architect chemiluminescent microparticle immunoassay. Serum CRP levels were estimated by Abbott Architect c-Systems by immunoturbidimetric method. The normal range of serum CRP is 0–5 mg/L [16].

Inclusion criteria

Laboratory (Reverse transcription polymerase chain reaction) confirmed COVID-19-positive patients.

Exclusion criteria

Pregnant women, children, and those unwilling to participate shall not be enrolled for the study.

RESULTS

Categorical variables were reported as counts and percentages. Group comparisons were made with the Chi-square test/Fisher's Exact test. Continuous data were given as mean, SD and range or median and inter-quartile range, as appropriate. Normality of quantitative data was checked by the measures of Kolmogorov–Smirnov tests of normality. For skewed data, comparisons for two groups (SpO_2 <94 and ≥94) were made by the Mann–Whitney test. For normally distributed data, a Student's t-test was applied to compare the two groups. For non-normally distributed data, a comparison based on the basis of severity of COVID-19 was made by Kruskal–Wallis test followed by Mann–Whitney test.

$p<0.05$ was considered statistically significant. Analysis was conducted using IBM SPSS statistics (version 22.0).

Out of 100 patients, 63% of patients have mild disease, 23% of patients have moderate disease, and 14% of patients have severe disease. The mean age of patients with mild disease was 61.4 years, with moderate disease was 64.2 years and severe disease was 62.4 years. It was

Table 10: Descriptive statistics in different SPO2 groups

SpO_2 groups	n	Mean	Standard deviation	Minimum	Maximum	Percentiles		
						25th	50th (Median)	75th
<94								
Blood Sugar	58	233.155	131.3728	88.0	672.0	136.000	195.500	301.000
T. bilirubin	58	0.8207	0.37075	0.30	2.40	0.6000	0.8000	0.9000
T. protein	58	6.533	1.1587	4.0	9.3	6.000	6.550	7.200
ALK phos	58	113.724	57.2240	32.0	286.0	78.750	96.500	127.000
SGOT	58	84.914	155.8371	18.0	884.0	38.000	46.500	72.000
SGPT	58	113.914	189.7405	27.0	1025.0	47.000	61.500	83.000
S. urea	58	52.328	26.6908	20.0	130.0	32.000	45.500	74.000
S. creatinine	58	1.1384	0.54956	0.60	3.90	0.8000	0.9000	1.2500
Na	58	137.397	5.3670	128.0	155.0	134.750	139.000	140.000
K	58	4.9559	4.84289	3.00	41.00	4.0750	4.3000	4.7000
D-dimer	58	599.052	665.2532	110.0	4660.0	212.250	465.000	739.000
Procal	58	0.2328	0.32198	0.01	1.78	0.0675	0.1000	0.2500
IL-6	58	16.5531	23.62613	0.06	141.00	1.9000	8.6000	19.0000
S. ferritin	58	444.752	369.2909	54.2	1650.0	171.000	340.800	598.500
CRP	58	42.3631	70.70195	6.00	392.00	16.1000	21.6000	40.6750
LDH	58	379.931	192.5845	101.0	952.0	240.250	338.000	490.000
CPK	58	128.414	178.6646	37.0	1289.0	64.750	82.000	121.000
SpO_2	58	82.086	9.8271	54.0	93.0	76.750	84.000	90.000
Duration of Symptoms (days)	58	7.84	4.400	1	18	4.00	7.00	11.25
SpO_2 groups	58	1.00	0.000	1	1	1.00	1.00	1.00
≥94								
Blood Sugar	42	200.245	92.0473	77.0	509.0	140.000	174.500	248.750
T. bilirubin	42	0.6769	0.22688	0.40	1.40	0.5000	0.6400	0.8000
T. protein	42	6.807	0.6564	5.5	8.0	6.200	6.800	7.250
ALK phos	42	108.452	30.8381	61.0	195.0	76.750	110.000	126.000
SGOT	42	71.814	63.6187	23.0	380.0	42.750	62.000	79.500
SGPT	42	104.633	125.1150	21.6	613.0	45.250	66.000	104.250
S. urea	42	45.119	26.5892	17.0	136.0	29.000	37.000	50.250
S. creatinine	42	1.1405	0.85883	0.60	5.10	0.8000	0.9500	1.1000
Na	42	136.119	4.1743	126.0	147.0	133.000	136.000	138.000
K	42	4.1981	0.47407	3.30	5.50	3.9550	4.2500	4.5000
D-dimer	42	381.571	289.6342	45.0	1330.0	205.000	287.500	447.500
Procal	42	0.8398	4.26952	0.03	27.80	0.0700	0.1100	0.1500
IL-6	42	10.2176	13.17785	0.10	59.90	1.9000	3.9500	14.4000
S. ferritin	42	862.067	1514.3549	3.5	7214.0	174.150	371.500	1025.000
CRP	42	35.5767	34.88162	4.90	171.00	18.9900	25.0000	37.8500
LDH	42	539.286	1099.9118	52.0	5372.0	202.000	312.500	403.250
CPK	42	218.381	300.3746	20.0	1257.0	67.000	88.500	285.000
SpO_2	42	95.762	1.6937	94.0	99.0	94.000	96.000	97.000
Duration of Symptoms (days)	42	4.83	3.028	1	16	2.75	4.50	6.25
SpO_2 groups	42	2.00	0.000	2	2	2.00	2.00	2.00

SGOT: Serum glutamic-oxaloacetic transaminase, SGPT: Serum glutamic-pyruvic transaminase, IL: Interleukin, CRP: C-reactive protein, LDH: Lactate dehydrogenase, CPK: Creatine phosphokinase

Table 11: Comparison of various parameters in different SpO2 groups Mann–Whitney test

Test statistics[a]				
Parameters	**Mann–Whitney U**	**Wilcoxon W**	**Z**	**Asymp. Sig. (2-tailed)**
Blood Sugar	1094.000	1997.000	−0.866	0.386
T. bilirubin	847.000	1750.000	−2.634	0.008**
T. protein	1055.000	2766.000	−1.140	0.254
ALK phos	1110.000	2821.000	−0.755	0.451
SGOT	1022.000	2733.000	−1.369	0.171
SGPT	1186.500	2897.500	−0.220	0.826
S. urea	979.500	1882.500	−1.667	0.096
S. creatinine	1093.000	1996.000	−0.883	0.377
Na	989.500	1892.500	−1.601	0.109
K	1004.000	1907.000	−1.499	0.134
D-dimer	957.500	1860.500	−1.820	0.069
Procal	1194.500	2905.500	−0.165	0.869
IL-6	1073.000	1976.000	−1.013	0.311
S. ferritin	1080.500	2791.500	−0.960	0.337
CRP	1086.500	2797.500	−0.918	0.358
LDH	1006.500	1909.500	−1.477	0.140
CPK	1095.500	2806.500	−0.856	0.392
SpO_2	0.000	1711.000	−8.533	<0.001**
Duration of Symptoms (days)	707.500	1610.500	−3.583	<0.001**

(Contd...)

Table 11: ***(Continued)***

[a]Grouping Variable: SpO_2 groups, SGOT: Serum glutamic-oxaloacetic transaminase, SGPT: Serum glutamic-pyruvic transaminase, IL: Interleukin, CRP: C-reactive protein, LDH: Lactate dehydrogenase, CPK: Creatine phosphokinase. Total bilirubin- T.bilirubin

observed that when a comparison was done between mild and severe disease patients, there was a significant difference between mean values of total bilirubin, serum sodium, serum potassium, D-dimer, SpO_2, and duration of symptoms. When the comparison was done between the various parameters in mild and moderate patients, the

mean levels of serum sodium, SpO_2, and duration of symptoms were significant. When comparison was done between moderate and severe patients, the mean levels of SpO_2 and duration of symptoms were significant. The number of patients having SpO_2 levels <94 is 58 with the mean age of patients is 63.38 years, whereas patients having SpO_2 levels more than or equal to 94 are 42 with the mean age of 60.63 years. In patients with SpO_2 <94, there were 33 males and 25 females and in patients with SpO_2 more than or equal to 94, there were 30 males and 12 females.

In patients with SpO_2 <94, 22 had mild disease, 22 had moderate disease and 14 had severe disease whereas in patients with SpO_2 more than or equal to 94, 41 had mild disease, and 1 had moderate disease. When a comparison was done between both the groups according to SpO_2, only the mean levels of serum total bilirubin were significant.

DISCUSSION

In the present study, it was observed that when patients were divided according to severity of disease, there was a significant difference in the mean levels of total bilirubin, serum sodium, serum potassium, d-dimer, SpO_2, and duration of symptoms in mild and severe group of patients, while there was no significant difference in the parameters such as IL-6, ferritin, CRP, PCT, LDH, and other routine biochemistry parameters. Whereas, when comparison was done in the mild and moderate group, only serum sodium levels were significant while there was no significant difference in other parameters. When a comparison was done between the moderate and severe group, no significant difference was found in any parameter except SpO_2 and duration of symptoms. In the various studies, it was observed that cytokines except IL-6, at the onset of disease showed the highest levels in serum. In this study, there was a significant increase in both the IL-6 and IL-10 levels in the seriously sick group as compared to the milder cases [17].

Various studies have revealed that unlike critically ill patients, the patients with mild infection had LDH values within the reference ranges [17,18]. The diminished serum LDH levels and CK were correlated with the viral mRNA elimination, which suggests that the diminution in the LDH or CK levels is likely to envisage a positive response to the itinerary of infection in COVID-19 patients [19]. Furthermore, as compared the mild patients, the elevated levels of alanine aminotransferase (ALT), aspartate aminotransferase, and total bilirubin are commonly seen in seriously ill patients, those showing frequent signs of liver dysfunction [20]. In a study conducted by Cai *et al*., it was observed that more than 90% of patients with abnormal hepatic tests were having a mild disease on admission and around 24% of them shown more than 3 times the upper limit elevation in ALT and gamma-glutamyl transferase during hospitalization [21].

In another study, the combination of increased high-sensitivity CRP and eosinopenia is used to distinguish between patients with pneumonia or a respiratory infection from the patients suspected of presenting COVID-19 [22]. In severe COVID-19 patients, the CRP was found to be significantly raised in the early phases of the infection before indications of any critical findings in CT scan. Hence, notably, CRP is an early predictor for severe disease and has been related with the development of the disease [23]. It has been observed in another study that CRP levels above 130 mg/L may be associated with an increase in mortality [24]. A successive increase in serum CRP or LDH could caution regarding progression to cytokine storm [25] (Tables 1-3).

Some other studies done on survivors and non-survivors of COVID-19, a significant increase in total bilirubin, CK, serum ferritin, white blood cell count, and IL-6 was seen in non-survivors as compared to survivors [26] (Table 4).

PCT produced mostly by extra-thyroid tissue such as the liver, pancreas, kidney, lung, intestine, and within leukocytes, and its levels may increase up to 100 ng/mL during severe bacterial, parasitic, and fungal infection having systemic manifestations whereas the synthesis of PCT is suppressed in extrathyroid tissue in the absence of bacterial infection (Table 5). The production of PCT can be amplified as a result of cytokines such as IL-6, TNF-a, and IL-1b and endotoxins [27] (Table 6). Similar to our study, in a study conducted by Gao *et al*., no significant association was found between PCT and the severity of disease [28] (Table 7). However, on the contrary, some studies have shown a significant association between the severity of disease and increased PCT levels [29,30] (Table 8).

In severe COVID-19 patients, serum albumin levels were significantly low, which does not correspond to the severity of hepatocellular damage, thus suggesting some other causes of hypoalbuminemia (Table 9). One of the causes of hypoalbuminemia may be the severe systemic inflammation seen in severe COVID-19, as it is common in many inflammatory diseases due to leakage of albumin into the interstitial space due to increased capillary permeability [31] (Tables 10 and 11). In admitted patients, the low levels of proteins and albumin have been associated with adverse outcome of the disease [32].

Derangement in renal parameters indicates inflammatory involvement and delayed recovery. The Electrolyte imbalance might be an early pointer of severity of the disease. In a study conducted by Prasad *et al*., they observed that the renal parameters, serum bilirubin were raised in non-survivors whereas total serum protein, albumin, and albumin-globulin ratio were markedly decreased. The risk for non-survival was increased with the elevation of serum levels of urea, creatinine, uric acid, sodium, and chloride [10].

Some studies had shown that as compared to CRP and LDH, ferritin had lower significance as a biomarker in monitoring and prediction of disease severity [12]. Whereas in other studies, serum ferritin was identified to be a significant marker for the prediction of severity of disease [20].

In our study, the levels of D-dimer were significantly higher in severe patients as compared to the patients having a mild disease. Several studies have found D-dimer as an efficient predictor for COVID-19 mortality and thus the severity of the disease [26]. The advantage of our study compared with other studies is that almost all the parameters (biomarkers) in the literature have been studied together in our study.

Limitations

However, there were a few limitations in our study, First, the sample size was small. Second, the confounding factors involved in various comorbid conditions were not taken into consideration. Third, all the routine biochemical investigations and markers were evaluated at the time of admission; hence the changes in parameters during the course of disease could not be evaluated.

CONCLUSION

In the present study, the various biochemical markers were studied according to the severity of the disease. It was observed that there was a significant difference between mean values of total bilirubin, serum sodium, serum potassium, d-dimer, SpO_2, and duration of symptoms in mild and severe group of patients. When a comparison was done between both the groups according to SpO_2, only the mean levels of serum total bilirubin were significant. We also could not establish significant associations of various other biomarkers, clinical and radiological findings in predicting the severity of coronavirus disease.

CONTRIBUTION OF AUTHORS

We declare that this work was done by the authors named in this article and all the liabilities pertaining to claims relating to the content of this article will be borne by the authors. Dr Pallavi Mahajan, Corresponding author, drafted the article and contributed in writing the article, data analysis, and interpretation of biochemical parameters. Dr Rachna

Sabharwal contributed in drafting, writing, and interpretation of biochemical parameters. Dr Rajesh Mahajan contributed in the drafting and writing discussion and interpretation of anesthetic parameters. Dr Fayaz Ahmad Wani contributed in providing patients data and writing the discussion for the article. Dr Animesh Mahajan contributed in data collection and compilation.

ETHICAL CLEARANCE

Ethical Clearance was obtained from the Institutional Ethical Committee of Government Medical College, Jammu.

CONFLICTS OF INTEREST

The authors declare that there is no conflicts of interest.

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None.

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