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Pathology

HEMATOLOGICAL AND BIOCHEMICAL FINDINGS OF PATIENTS WITH SARS-COV-2 INFECTION- AN OBSERVATIONAL STUDY FROM CENTRAL INDIA.

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ABSTRACT **Background-** in the present research various haematological and biochemical parameters of COVID-19 patients belonging to Central India population have been studied which can be useful in diagnosing the COVID-19 disease and to determine its severity. **Material & methods-** This observational study included secondary data of 700 adult subjects diagnosed to have COVID-19 infection. Subjects were classified as those having mild, moderate and severe disease based on the findings of Chest CT. Levels of D-dimer, C-reactive protein, ferritin concentration, haemoglobin concentration, total leucocyte count, differential leucocyte count, neutrophil:lymphocyte ratio, blood group, Prothrombin time, INR, aPTT, fibrin degradation product, blood glucose, HbA1c, PCV, WBC count, platelet count, ESR, IL-6, SGOT, SGPT, LDH, urea, creatinine, sodium, potassium was analysed. **Results-** Mean age of the subjects was 46.3±16.042 years (range- 18.0 to 94.0 years). Male:female ratio was 1.9:1. Diabetes was present in 40.3% and 28.1% subjects had diabetes and hypertension both. CT chest revealed that maximum (40.0%) patients were having moderate, 38.6% had mild and 21.4% had severe COVID-19 disease. On comparing the mean values of different parameters amongst patients with different severity grade, D-dimer, FDP, urea, LDH, potassium and chlorine concentration were found to differ significantly between different severity grades (p value <.05). **Conclusion-** Based on the findings of the present study, it can be concluded that the levels of D-dimer, C-reactive protein, ferritin concentration, haemoglobin concentration, total leucocyte count, differential leucocyte count, neutrophil:lymphocyte ratio, blood group, Prothrombin time, INR, aPTT, fibrin degradation product, blood glucose, HbA1c, PCV, WBC count, platelet count, ESR, IL-6, SGOT, SGPT, LDH, urea, creatinine, sodium, potassium varying in different amount from normal range amongst patients with Covid-19 disease. There is a significant association between severity of disease and D-dimer, FDP, ferritin and potassium levels.

KEYWORDS : COVID-19; Coronavirus; D-dimer: fibrinogen degradation products; Hematological parameters.

INTRODUCTION

The COVID-19 disease caused by highly pathogenic severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has been declared the global pandemic in 2020.[1] Clinical manifestation ranges from asymptomatic carrier state to severe respiratory distress, multiple organ dysfunction and death.[2]Corona virus have high affinity towards mucosa of the respiratory system and thus mainly effects respiratory system [3] Exchange of droplets primarily accounts for its transmission.[1] Patients with COVID-19 infection usually present with symptoms of fever, cough and dyspnea.[4] Few patients may also report myalgias/fatigue, rhinorrhoea, sore throat, headache, and diarrhoea.[4]

Lack of proper treatment at appropriate time can lead to complications such as respiratory failure, septic shock and multiple organ failure in severe cases.[5]The accurate diagnosis of COVID-19 infection is based on clinical features, radiological findings and laboratory findings. Laboratory findings amongst COVID-19 patients include lymphocytopenia and raised CRP (C- reactive protein) level, in some cases, elevated D-dimer, elevated ferritin, thrombocytopenia, elevated fibrinogen, prolonged PT, elevated lactate dehydrogenase, elevated factor VIII, circulating prothrombotic microparticles, hyperviscosity and increased formation of neutrophil extracellular traps (NETs).[6] Normal or prolonged prothrombin time (PT) and active partial thromboplastin time (aPTT) are commonly found.[1] The hematologic profile of the patients mainly depends on the severity of the disease.[3] Hemoglobin concentration values have been found to be the determinants for the differential diagnosis between community-acquired pneumonia and COVID-19 disease.[7] Low platelet count, elevated C-reactive protein (CRP) and LDH have been found to have significant association with mortality amongst patients with COVID-19 disease.[8] CRP levels are elevated accompanying the lung lesions and therefore, it reflects the severity of COVID-19 disease.[9] CRP level >41.8 mg/L has been suggested as an independent risk factor for progression in the early stage COVID-19 patients.[10] White blood cell count of patients can be below 4×109 /L (Leucopenia). Lower levels of absolute lymphocyte count (ALC) have been noticed in patients admitted to ICU. Lymphocyte count can be less than 1.0×109 /L.[3] Neutrophil/lymphocyte ratio has also been suggested as an independent risk factor for the severe COVID-19 infection.[11] In these patients excess formation of pro-inflammatory cytokines leads to a phenomenon referred to as cytokine storm. Cytokine storm resulting in vascular hyper-permeability, acute respiratory distress syndrome (ARDS) and even organ failures have been noticed in many patients with COVID-19 infection.[3] Amongst children with mild and severe COVID-19 disease, procalcitonin (PCT), CRP and LDH levels have been demonstrated to be significantly elevated. Elevation of CK-MB could predict early cardiac injury in such children.[12] Various parameters can be useful in determining the occurrence and severity of COVID-19 disease. Also, geographical variations in the values of many parameters have been observed. [3] Thus, in the present research various haematological and biochemical parameters of COVID-19 patients belonging to Central India population have been studied which can be useful in diagnosing the COVID-19 disease and to determine its severity.

MATERIAL & METHODS

Study design, study population, sample size, sampling technique

This observational study was conducted in the department of Pathology, Sri Aurobindo Medical College and PG institute, Indore, India after obtaining approval from the institutional ethics committee. The study included secondary data of 700 COVID-19 positive adult subjects who visited the OPD/IPD of the Sri Aurobindo Medical College and PG Institute, Indore (M.P) over the period of 1 year (from January 2021 to December 2021). Laboratory reports of only those subjects were procured who were diagnosed via RT-PCR to have COVID-19 infection Enrolment of subjects in the study was done using convenience sampling technique.

Parameters studied

The confirmed cases of COVID-19 disease were classified as those having mild, moderate and severe disease based on the findings of Chest CT.[13]

The blood parameters taken into consideration were D-dimer, C-reactive protein, ferritin concentration, haemoglobin concentration, total leucocyte count, differential leucocyte count, neutrophil:

lymphocyte ratio, blood group, Prothrombin time, INR, aPTT, fibrin degradation product, blood glucose, HbA1c, PCV, WBC count, platelet count, ESR, IL-6, SGOT, SGPT, LDH, urea, creatinine, sodium, potassium was analysed. Levels of various parameters were classified as normal, decreased or increased based on the normal range mentioned in the standard textbooks.

A wide variety in the normal range of various biochemical and haematological parameters have been reported in literature. In this study, the normal range of different parameter considered in this study has been presented in table 1:[14][15][16][17]

Table 1. Normal range of various parameters.

Parameters	Range
HbA1c	<6.5%
Hemoglobin concentration (Men) (gm/l)	150 ± 20 g/l
Hemoglobin concentration (Women) (gm/l)	135 ± 15 g/l
Total leucocyte count (per cubic mm)	4 - 10 x 109/l
Neutrophil count (%)	40-80%
Lymphocyte count (%)	20-40%
Monocyte count (%)	2-10%
Eosinophil count (%)	1-6%
Basophil count (%)	<1-2%
Neutrophil:lymphocyte ratio	0.78 to 3.53
Platelet count	280 ± 130 x 109/l
Erythrocyte sedimentation rate (men) (mm/hour)	15-20 mm/hour
Erythrocyte sedimentation rate (women) (mm/hour)	15-20 mm/hour
Prothrombin time (seconds)	10-13 seconds
INR	0.8–1.1
aPTT (seconds)	26-40 seconds
D-dimer (mg/l)	<200 mg/l
Fibrinogen degradation product (µg/ml)	<10 µg/ml
SGOT (U/L)	5-40 U/L
SGPT (U/L)	7-56 U/L
Urea (mg/dl)	6-24 mg/dL
Creatinine (men) (mg/dl)	0.74 to 1.35 mg/dL
Creatinine (women) (mg/dl)	0.59 to 1.04 mg/dL
Serum Ferritin (men) (µg/l)	15-300 µg/l
Serum Ferritin (women) (µg/l)	15-200 µg/l
C-reactive protein (mg/L)	<10 mg/L
LDH (IU/L)	105 to 333
Blood glucose (fasting) (mg/dl)	<126 mg/dl
Blood glucose (random) (mg/dl)	<200 mg/dl
Sodium concentration (mEq/L)	135-145 mEq/L
Potassium concentration (mEq/L)	3.6-5.2 mEq/L
Chlorine concentration (mEq/L)	96-106 mEq/L

gm- gram, l- litre, mm- millimetre, mg- milligram, µg- microgram, IU- International unit, dl- decilitre, mEq- milliequivalent

The data was recorded into a pre-designed proforma. Data was analyses using SPSS (Statistical Package for Social Sciences) 21.0 version, IBM, Chicago. Data were analysed for probability distribution using Shapiro-wilk test. Descriptive statistics was performed. Data were described as mean (Standard Deviation) and number and percentage. Inter-group comparison was done using One-way ANOVA. Association between the variable was assessed using Chi-square test. P value <.05 was considered statistically significant.

RESULTS

The study included 700 patients with the mean age of 46.3±16.042 years (18.0 to 94.0 years). Most of the patients belonged to the age group of 18-30 years (20.7%). The number of male patients was more than the number of female patients (65.7% vs 34.3%). At the time the study was conducted only 28.3% patients were vaccinated against SARS Cov-2 virus. The most common comorbidity present amongst the study subjects was only diabetes (40.3%) and 28.1% subjects had diabetes and hypertension both. CT chest revealed that maximum (40.0%) patients were having moderate, 38.6% had mild and 21.4% had severe COVID-19 disease. The mean ±standard deviation values of various haematological and biochemical parameters have been presented in table 2 and table 3. On comparing the mean values of different parameters amongst patients with different severity grade, D-dimer, FDP, urea, LDH, potassium and chlorine concentration were found to differ significantly between different severity grades (p value <.05).

With the increase in severity grade, there was a significant increase in D-dimer, FDP, urea, LDH, potassium concentration. [Table 2]

Table 2. Comparison of mean values of various hematologic parameters amongst study subjects with different severity of disease.

Parameter	Severity	Mean	Standard deviation	F value	P value[a]
Hemoglobin concentration (gm%)	Mild	11.82	2.111	.112	.894
	Moderate	11.90	2.142		
	Severe	11.86	2.165		
	All subjects	11.86	2.132		
Total leucocyte count (per cubic mm)	Mild	9090.72	5952.687	1.460	.233
	Moderate	8988.82	5127.021		
	Severe	11880.73	7598.303		
	All subjects	9647.82	8078.286		
Neutrophil count (%)	Mild	11.718	.713	.426	.653
	Moderate	12.176	.727		
	Severe	13.727	1.120		
	All subjects	74.11	12.344		
Lymphocyte count (%)	Mild	10.915	.664	.024	.977
	Moderate	11.590	.692		
	Severe	13.375	1.092		
	All subjects	19.87	11.731		
Monocyte count (%)	Mild	3.182	.193	2.900	.056
	Moderate	3.338	.199		
	Severe	3.020	.246		
	All subjects	4.91	3.221		
Eosinophil count (%)	Mild	1.686	.102	.588	.556
	Moderate	1.693	.101		
	Severe	1.504	.122		
	All subjects	1.05	1.01		
Basophil count (%)	Mild	.000	.000	-	-
	Moderate	.000	.000		
	Severe	.000	.000		
	All subjects	.000	.000		
Neutrophil: lymphocyte ratio	Mild	5.93	5.113	1.351	.260
	Moderate	6.27	5.552		
	Severe	7.00	6.382		
	All subjects	6.30	6.106		
Platelet count	Mild	2.29	1.281	.671	.512
	Moderate	2.42	1.324		
	Severe	2.41	1.340		
	All subjects	2.37	1.311		
Erythrocyte sedimentation rate (mm/hour)	Mild	41.53	19.463	.565	.569
	Moderate	40.98	20.569		
	Severe	43.16	21.730		
	All subjects	41.66	20.395		

[a]One-way ANOVA. *p value <.05 was considered statistically significant.

gm- gram, l- litre, mm- millimetre, mg- milligram, µg- microgram, IU- International unit, dl- decilitre, mEq- milliequivalent

Table 3. Comparison of mean values of various hematologic and biochemical parameters amongst study subjects with different severity of disease.

Parameter	Severity	Mean	Standard deviation	F value	P value[a]
HbA1c	Mild	7.07	1.858	.240	.787
	Moderate	7.09	1.800		
	Severe	7.19	1.733		
	All subjects	7.10	1.807		
Prothrombin time (seconds)	Mild	16.40	5.607	.222	.801
	Moderate	16.50	5.998		
	Severe	16.80	6.174		
	All subjects	16.53	5.883		
INR	Mild	1.10	.197	.359	.659
	Moderate	1.10	.218		

	Severe	1.12	.244		
	All subjects	1.11	.216		
aPTT (seconds)	Mild	29.34	7.350	1.077	.341
	Moderate	30.20	6.730		
	Severe	29.96	6.934		
	All subjects	29.81	7.019		
D-dimer (ng/dl)	Mild	252.28	238.057	143.960	.001*
	Moderate	615.51	483.481		
	Severe	1142.98	841.701		
	All subjects	588.44	413.237		
Fibrinogen degradation product (ng/dl)	Mild	495.34	467.182	140.192	.001*
	Moderate	1203.72	912.740		
	Severe	2359.73	1908.690		
	All subjects	1178.21	1093.458		
SGOT (U/L)	Mild	54.80	53.832	2.245	.107
	Moderate	49.09	44.887		
	Severe	61.34	51.277		
	All subjects	53.92	51.870		
SGPT (U/L)	Mild	50.69	43.719	2.451	.087
	Moderate	47.96	46.128		
	Severe	59.68	45.220		
	All subjects	51.53	43.015		
Urea (mg/dl)	Mild	39.05	21.111	3.127	.044*
	Moderate	39.92	19.203		
	Severe	44.10	21.219		
	All subjects	40.48	20.455		
Creatinine (mg/dl)	Mild	1.00	.601	1.024	.360
	Moderate	.94	.494		
	Severe	.94	.554		
	All subjects	.96	.550		
Ferritin (ng/ml)	Mild	699.13	472.035	.063	.939
	Moderate	655.51	531.491		
	Severe	707.56	589.852		
	All subjects	683.49	513.209		
C-reactive protein (mg/dl)	Mild	3.00	1.877	1.851	.158
	Moderate	7.44	4.538		
	Severe	13.49	6.154		
	All subjects	7.02	3.799		
LDH (IU/L)	Mild	355.58	206.113	3.083	.046*
	Moderate	390.66	305.165		
	Severe	420.54	274.981		
	All subjects	383.53	265.053		
Blood glucose (mg/dl)	Mild	144.26	41.696	.864	.422
	Moderate	145.60	41.095		
	Severe	149.74	41.357		
	All subjects	145.97	41.376		
Sodium concentration (mEq/L)	Mild	135.14	16.325	.051	.950
	Moderate	135.59	16.233		
	Severe	135.43	17.501		
	All subjects	135.38	16.525		
Potassium concentration (mEq/L)	Mild	4.04	.704	3.539	.030*
	Moderate	4.06	.634		
	Severe	4.22	.800		
	All subjects	4.09	.702		
Chlorine concentration (mEq/L)	Mild	110.17	11.888	3.608	.028*
	Moderate	113.01	12.844		
	Severe	112.28	13.637		
	All subjects	111.76	12.709		

[a]One-way ANOVA. *p value <.05 was considered statistically significant.

gm- gram, l- litre, mm- millimetre, mg- milligram, µg- microgram, IU- International unit, dl- decilitre, mEq- milliequivalent

On assessing the association between severity of disease and different parameters, it was found that there was a significant association between D-dimer values and CT grade [Chi square value-216.758, df-2, p value- .001]. The proportion of patients with increased D-dimer was significantly more in grade III patients. [Figure 1] Also, the proportion of patients with increased FDP was significantly more in grade II and III patients indicating that there was a significant association between FDP and CT grade [Chi square value- 217.664, df-2, p value- .001]. [Figure 2] Ferritin levels were also found to have significant association with CT grade. [Chi square value- 14.591, df-4, p value- .006]. [Figure 3]

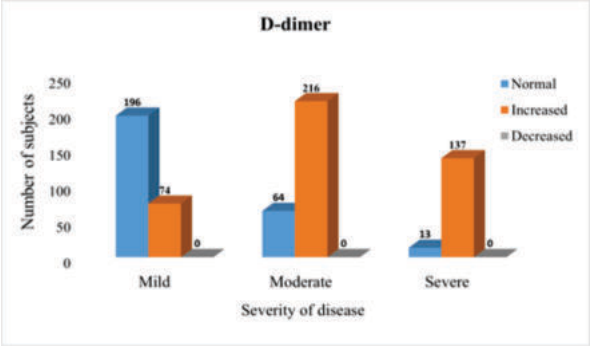

Figure 1. Distribution of study subjects based on disease severity and D-dimer.

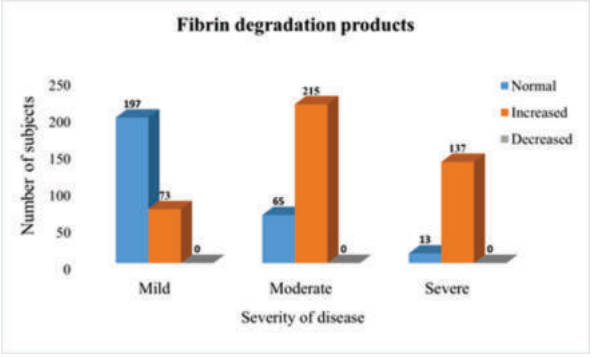

Figure 2. Distribution of study subjects based on disease severity and FDP.

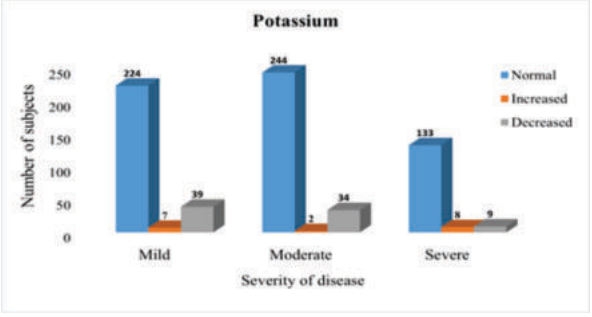

Figure 3. Distribution of study subjects based on disease severity and potassium concentration.

DISCUSSION

The accurate diagnosis of COVID-19 infection is based on clinical features, radiological findings and laboratory findings. In this study we have provided information on the various hematological and biochemical parameters amongst patients with COVID-19 disease from Central India. Present study included 700 patients aged 18-94 years. This conforms to fact that COVID-19 disease had affected adult individuals of all age groups.[18] In the present study, the number of male patients was more than the number of female patients (65.7% vs 34.3%). **Abate BB et al. (2020)** in their metanalysis reported prevalence of symptomatic COVID-19 to be higher in men than in women. High prevalence of smoking and alcohol consumption amongst men have been thought to contribute towards the high prevalence of COVID-19 among men.[19] Previous researches have reported a wide range of prevalence of diabetes (9.6% to 40.8%) and cardiovascular disease (2.5% to 15.0%) amongst patients with

COVID-19 disease. The importance of existence of diabetes and hypertension amongst these patients lies in the fact that these comorbid conditions can results in severe COVID-19 disease.[20] In our study, diabetes alone (40.3%) was found to be more prevalent as compared to the hypertension alone (9.0%), 28.1% patients were found to have both diabetes as well as hypertension.

Hemoglobin

COVID-19 patients have been reported to show impaired hemoglobin biosynthesis. SARS-CoV-2 proteins interacts with hemoglobin molecules, leading to hemoglobin dysfunction, tissue iron overload and disrupted heme metabolism.[21] In the present study, a high percentage (63.4%) of patients have been found to show decreased hemoglobin levels.

N/L Ratio

Results of our study showed high NLR ratio amongst majority (60.1%) of patients with COVID-19 disease. Similar results were reported by **Anurag A et al. (2020)**[22], **Gothwal SK et al. (2021)**[23], **Kazancioglu S et al. (2020)**[24], **Qin C et al. (2020)**[25]. NLR has been found to be an independent risk factor for the severe COVID-19 disease.[26]

ESR

ESR is a marker of inflammation. Patients with COVID-19 disease have shown elevated levels of ESR.[27] Our study showed elevated ESR values in 91.4% patients. The mean ESR reported was 41.66±20.395 mm/hour. Similar findings were reported by **An XS et al. (2020).**[28]

C-reactive protein

CRP levels are indicative of level of inflammation. CRP levels can activate the complement and enhance phagocytosis as a part of immune response. Patients with severe pneumonia have been found to have high CRP levels. Amongst patients with COVID-19 disease, CRP levels have been found to be positively correlated with lung lesion and disease severity. [10] In the present study a large number of patients (55.7%) were found to have elevated CRP levels. Elevated CRP levels were also reported by **Ali AM et al. (2021).**[29]

Fibrinogen Degradation Product

Fibrinogen is an acute phase protein. It is synthesized in large amount by liver in response to IL-1 and IL-6 derived stimulation. It is involved in fibrin formation in coagulation mechanism. Levels of fibrinogen are used for diagnosis of DIC. Hence, fibrinogen has been considered the subject of investigation in the Covid-19 pandemic, due to the close relationship between Covid-19 and DIC.[30] Present study showed that there was also increase in concentration of fibrinogen degradation products which indicted decrease in fibrinogen concentration. **Hayıroğlu Mİ et al. (2020)** also reported increase in D-dimer and decrease in fibrinogen concentration among patients with COVID-19 disease.[30]

D-dimer

D-dimer is a soluble plasmin-mediated degradation product of fibrin, which is produced on fibrinolysis.[30] D-dimer level are being assessed in patients to detect thrombosis. Previous research has reported an elevated levels of D-dimer and fibrinogen concentrations in the early stages of COVID-19 disease. It has also been observed that a 3 to 4-fold rise in D-dimer levels is associated with poor prognosis in these patients.[31] **Ali AM et al. (2021)** had reported elevated levels of D-dimer.[29] In the present study, the D-dimer levels were found to be raised in 61.0% patients. The mean D-dimer level among the study subjects was 588.44 ng/dl. In the present study the D-dimer values significantly higher in patients with CT grade III compared to grade II and grade I. This conforms to the fact that elevation in levels of D-dimer is significantly associated with the severity of the disease.[32]

Potassium

SARS-CoV-2 virus interact with the renin–angiotensin–aldosterone system[33] and therefore, hypokalaemia can be found in high percentage of COVID-19 patients, **Nasomsong W et al. (2021)** reported hypokalaemia in 94.4% patients. Hypokalaemia develops earlier in the course of COVID-19 disease, without existing extra-renal potassium loss.[34] **Alfano G et al. (2021)** reported hypokalaemia in 41% hospitalized COVID-19 patients.[33] In the present study, concentration of potassium was within normal range in most of the subjects (85.9%) and hypokalaemia was seen in 11.7% patients. The difference in the findings of this study with our study can be attributed to the fact that in our study included laboratory parameters of all patients irrespective of their hospitalization status. In the present study, the mean potassium concentration was found to be 4.09 mEq/L. **Alfano G et al. (2021**) also reported only mild decrease in potassium levels with mean serum potassium levels of 3.1±0.1 meq/L.[33] It is important to correct hypokalaemia at its earliest as it is associated with potentially life threatening complications such as cardiac dysrhythmias, rhabdomyolysis and paralysis.[33]

Sodium

In the present study hyponatremia was seen in 36.0% patients and hypernatremia was seen in 21.9% patients. Hyponatremia and hypernatremia both have been found to be associated with renal insufficiency. It is important to determine and correct sodium levels in COVID-19 patients as mortality rate have been found to be higher among patients with sodium balance disorders compared to normonatremia.[35]

LDH

LDH is an intracellular enzyme involved in anaerobic glycolysis. It catalyzes the oxidation of pyruvate to lactate. Elevated serum LDH level have been identified as an independent indicator of severity and mortality in patients with COVID-19.[36] In the present study, LDH levels were found to be increased in 14.3% patients. The mean concentration of LDH was found to be 383.53±265.053 (IU/L). LDH levels found to be significantly high in patients with CT grade III. Similar findings were reported by **Dong X et al. (2020).**[37]

SGOT & SGPT

In the present study the SGOT and SGPT levels were found to be elevated in 30.8% and 36.8% patients respectively. Previous research has reported that prevalence of ALT elevations among patients with COVID،19 ranged between 4% and 39% and prevalence of AST elevations ranged between 4% and58%.[38]

Creatinine

Present study revealed elevated levels of plasma creatinine levels in 15.6% patients and elevated urea levels in 41.0% patients. The mean creatinine concentration was found to be 0.96±0.550 mg/dl and mean urea concentration was found to be 40.48 ± 20.455 mg/dl. Similar findings were reported by **Mahmoudi H et al. (2020)**.[39] They have reported that mean urea concentration at duration of 2, 3 and 4 days after the onset of viral infection were 34.75±0.10 and 37.64±0.32, 39.81±0.10, 42.56±0.35 mg/dl, respectively.[39] It has been suggested that assessment of creatinine and urea levels at the onset of the disease and after (2 to 4 days) represents impaired kidney function is occurs in COVID-19 patients.[39]

Unlike other studies, in the present study the levels of many laboratory parameters were not found to differ significantly between different severity grades of disease. this could be explained by the fact that severity of disease was determined on the basis of CT score only and not on the basis of CT score and need of ICU admission.

Limitations of the study,

Major limitation of the study was that in this study only the laboratory parameters have been studied and they had not been related with the clinical signs and symptoms and the ongoing treatment. In the present study, severity of disease was determined on the basis of CT score only and not on the basis of CT score and need of ICU admission. In the present study outcome (cure/mortality) was not taken into consideration. In the present study, history of treatment against COVID-19 disease was considered.

It is a single-center, non-externally validated, cross-sectional study. Bias associated with the study design could not be removed.

CONCLUSION

Based on the findings of the present study, it can be concluded that the levels of D-dimer, C-reactive protein, ferritin concentration, haemoglobin concentration, total leucocyte count, differential leucocyte count, neutrophil:lymphocyte ratio, blood group, Prothrombin time, INR, aPTT, fibrin degradation product, blood glucose, HbA1c, PCV, WBC count, platelet count, ESR, IL-6, SGOT, SGPT, LDH, urea, creatinine, sodium, potassium varying in different amount from normal range amongst patients with Covid-19 disease. There is a significant association between severity of disease and D-dimer, FDP, ferritin and potassium levels.

Conflict of interest None

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