Biochemical Communication

Alterations in Various Biochemical Parameters Among Covid-19 Patients: An Observational Retrospective Analysis

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ABSTRACT

Novel coronavirus causing the pandemic infectious disease termed as COVID-19 is characterized by respiratory illness which may lead on to acute respiratory distress syndrome. Ferritin is a key mediator of immune dysregulation leading on to cytokine storm. Alterations in various biochemical parameters have been widely reported in COVID-19. Early identification of effective biomarkers to assess the severity of this disease is essential. Our study was aimed to evaluate the variations in the routinely analysed biochemical parameters and their association with ferritin levels among COVID patients. The study participants consisted of 270 members among which 149 were COVID positive and 121 were negative. Analysis of the routine biochemical parameters as well as ferritin level were carried out. Among the 149 positive cases, 84 (56.4%) were mild positive with ferritin levels <500ng/ml and 65 (43.6%) were severe positive with ferritin levels >500ng/ml. We reported significant increase in serum ferritin levels in severe positive samples (1449.84 ± 249.47) compared to mild positive samples (230.04 ± 17.41). We observed increased levels of total bilirubin in 12.7%, direct bilirubin in 16.8%, indirect bilirubin in 8.7%, AST in 65.8%, ALT in 44.3%, ALP in 9.4%, GGT in 51.7%, urea in 18.4%, creatinine in 14.3%, BUN in 18.4% and decreased levels of total protein and albumin in 23.5% positive patients compared to negative patients. Ferritin and its associated biochemical parameters act as predictors of COVID severity. These biochemical alterations suggest the significance of early risk assessment and monitoring of COVID patients.

KEY WORDS: BIOCHEMICAL PARAMETERS, COVID-19, ELECTROLYTE ABNORMALITIES, FERRITIN, LIVER FUNCTION TESTS.

INTRODUCTION

COVID-19 which has been officially declared as a pandemic by the World Health Organization is characterised by respiratory illness which may progress on to severe pneumonia and acute respiratory distress syndrome (ARDS) (Park et al. 2020; Mbarka et al. 2020; Ashour et al. 2020). Since the outbreak of the coronavirus pandemic several abnormalities in various biochemical parameters have been reported but their clinical implications need to be investigated. Several meta-analyses have thrown light on the importance of serial assessment of biochemical parameters namely ferritin, lactate dehydrogenase (LDH), total bilirubin, total protein, albumin, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), urea, creatinine and electrolytes in evaluation of risk. Ferritin is a key mediator of immune dysregulation via direct immunosuppressive and pro-inflammatory effects contributing to cytokine storm (Ashour et al. 2020; Henry et al. 2020).

Ferritin may be considered as a strong discriminator for potential progression to critical illness of COVID-19 (Zhou et al. 2020). In view of the limited resources available, the early diagnosis of severe COVID-19 is of great importance to reduce morbidity and mortality. Assessing serum ferritin levels along with identification of derangements of other biochemical parameters during hospitalization can help to identify at risk individuals with COVID-19. The advantage of utilizing these parameters for effective triaging is the availability of highly standardized automated analyzers and reagent kits which offer rapid, reliable, reproducible results which are also economical (Henry et al. 2020). This study is proposed to identify potential biochemical laboratory markers which can be used to assess the progression of

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disease from mild to severe form and help in timely triaging of the patients.

MATERIAL AND METHODS

This retrospective study included patients of SRM Medical College Hospital and Research Centre, SRM Institute of Science and Technology, SRM Nagar, Kattankulathur, Kanchipuram, Chennai, Tamil Nadu, India. This study was carried out between the months of May and August 2020. Patients who tested positive for COVID-19 by RT-PCR testing were included as the study group and those who tested negative for COVID-19 by RT-PCR testing on admission were chosen as the control group. The study protocol was approved by the Scientific and Ethical committees of our Institution (IEC No: 1977/IEC/2020). All the samples were collected and processed as per the ICMR safety guidelines.

Table 1. Age Group of Participants

Age group	Positive (n-149)	Negative (121)
<20	0	6 (4.96 %)
20-29	13 (8.72 %)	21 (17.36%)
30-39	33 (22.15%)	39 (32.23%)
40-49	33 (22.15%)	22 (18.18 %)
50-59	25 (16.78 %)	23 (19.01 %)
≥60	45 (30.20 %)	10 (8.26 %)

Table 2. Chi Square Analysis Between Gender And Ferritin

Ferritin levels (ng/ml)	Positive (N-149)		Negative (N-121)	
	Male (N-111)	Female (N-38)	Male (N-75)	Female (N-46)
<500 ng/ml	52 (46.8%)	32 (84.2%)	63 (84.0%)	44 (95.7%)
>500 ng/ml	59 (53.2%)	6 (15.8%)	12 (16.0%)	2 (4.3%)

The parameters of renal function test, liver function test, serum electrolytes and lactate dehydrogenase were estimated using Beckman Coulter Auto analyser. Ferritin level was measured in Enhanced CLIA-Vitros ECi immunoanalyzer. After completion of estimations, the sample processing area and instruments were sterilized using hypochlorite solution and alcohol-based sanitizer. The biohazard materials used during the sample processing were disposed appropriately as per instructions in the safety guidelines. Based on the ferritin values obtained, the patients were categorized as having mild or severe coronavirus disease using a cut-off value of 500 ng/ml (Lin et al. 2020). The statistical analysis of study parameters was done using SPSS software (version 22).

Table 3. Analysis Of Positive And Negative Samples

Parameters	Positive		Negative		Significance P-value
	N	Mean ± SEM	N	Mean ± SEM	
Ferritin (ng/ml)	149	762.16 ± 119.62	121	264.99 ± 31.95	0.000***
LDH (U/L)	110	359.94 ± 22.76	105	272.66 ± 9.74	0.001**
Urea (mg/dl)	149	38.96 ± 3.56	121	24.37 ± 1.40	0.001**
Creatinine (mg/dl)	149	1.55 ± 0.21	121	0.76 ± 0.04	0.001**
BUN (mg/dl)	149	18.20 ± 1.66	121	11.38 ± 0.66	0.001**
Sodium (mmol/l)	146	134.84 ± 0.99	115	137.50 ± 0.28	0.021*
Potassium (mmol/l)	145	4.09 ± 0.05	115	3.94 ± 0.03	0.017*
Chloride (mmol/l)	145	100.67 ± 0.39	115	102.58 ± 0.33	0.000***
Bicarbonate (mmol/l)	145	24.19 ± 0.59	115	24.62 ± 0.28	0.544
Total bilirubin (mg/dl)	149	0.69 ± 0.03	120	0.59 ± 0.03	0.023*
Direct bilirubin (mg/dl)	149	0.20 ± 0.02	120	0.16 ± 0.02	0.049*
Indirect bilirubin (mg/dl)	149	0.49 ± 0.03	120	0.43 ± 0.02	0.092
Total protein (g/dl)	149	7.03 ± 0.08	120	7.34 ± 0.06	0.003**
Albumin (g/dl)	149	3.88 ± 0.06	120	4.08 ± 0.05	0.013*
Globulin (g/dl)	149	3.23 ± 0.04	120	3.25 ± 0.03	0.703
AG ratio	149	1.23 ± 0.02	121	1.26 ± 0.02	0.248
AST (IU/L)	149	59.16 ±11.68	120	35.37 ± 2.02	0.071
ALT (IU/L)	148	50.01 ± 7.40	120	30.08 ± 1.80	0.018*
ALP (IU/L)	148	88.21 ± 6.83	120	84.21 ± 3.34	0.625
GGT (U/L)	149	51.68 ± 4.08	120	40.50 ± 3.63	0.047*

Significance: *P<0.05, **P<0.01, ***P<0.001

Table 4: Unpaired 'T Test Between Mild (Ferritin <500ng/Ml) And Severe (Ferritin >500ng/Ml) Positive Groups

Parameters	Mild positive		Severe positive		Significance
	N	Mean ± SEM	N	Mean ± SEM	P-value
Ferritin (ng/ml)	84	230.04 ± 17.41	65	1449.84 ± 249.47	0.000***
LDH (U/L)	69	285.97 ± 13.25	41	484.41 ± 51.65	0.000***
Urea (mg/dl)	84	27.70 ± 2.23	65	53.51 ± 7.27	0.000***
Creatinine (mg/dl)	84	1.18 ± 0.22	65	2.02 ± 0.39	0.048*
BUN (mg/dl)	84	12.93 ± 1.04	65	24.99 ± 3.40	0.000*
Sodium (mmol/l)	82	136.38 ± 0.43	64	132.88 ± 2.19	0.081
Potassium (mmol/l)	82	4.09 ± 0.07	63	4.09 ± 0.08	0.977
Chloride (mmol/l)	82	101.79 ± 0.49	63	99.21 ± 0.58	0.001**
Bicarbonate (mmol/l)	82	25.01 ± 0.94	63	23.11 ± 0.59	0.112
Total bilirubin (mg/dl)	84	0.60 ± 0.04	65	0.80 ± 0.05	0.004**
Direct bilirubin (mg/dl)	84	0.17 ± 0.02	65	0.25 ± 0.03	0.028*
Indirect bilirubin (mg/dl)	84	0.43 ± 0.04	65	0.56 ± 0.03	0.023*
Total protein (g/dl)	84	7.19 ± 0.11	65	6.81 ± 0.11	0.017*
Albumin (g/dl)	84	4.08 ± 0.08	65	3.64 ± 0.07	0.000***
Globulin (g/dl)	84	3.28 ± 0.05	65	3.17 ± 0.07	0.203
AG ratio	84	1.26 ± 0.03	65	1.18 ± 0.03	0.047*
AST (IU/L)	84	40.44 ± 5.30	65	83.35 ± 25.70	0.068
ALT (IU/L)	83	33.08 ±3.25	65	71.61 ± 15.99	0.009**
ALP (IU/L)	83	79.23 ± 3.81	65	99.68 ± 14.72	0.138
GGT (U/L)	84	39.61± 4.16	65	67.29 ± 7.25	0.001**

Significance: *P<0.05, **P<0.01, ***P<0.001

Student's test was used for comparison of parameters between the groups. Pearson's correlation was utilized to assess the association between the various parameters and multiple comparison analysis was done using ANOVA.

RESULTS AND DISCUSSION

The study participants included 270 symptomatic patients who underwent COVID testing on the day of admission. Among them 149 (55%) tested positive and 121 (45%) were negative. Among the 149 positive cases, 111 (74.5%) were males and 38 (25.5%) were females. Amidst the 121 negative cases, 75 (62.0%) were males and 46 (38.0%) were females. We observed that males were more prone to COVID infection as compared to females. The maximum numbers of positive cases were found in the age group of above 60 years (Table 1).

The lactate dehydrogenase levels in adults ranges from 140-280 U/L. We found 44 (40%) of Covid positive patients having LDH in the normal range whereas 64 (58.2%) had LDH levels greater than 280 U/L.

We observed significant increase in the levels of ferritin, lactate dehydrogenase, urea, creatinine, BUN, potassium, total bilirubin, direct bilirubin, alanine transaminase, gamma glutamyl transferase and significant decrease in the levels of sodium, chloride, total protein, albumin between positive and negative COVID samples (Table 3).

We observed significant increase in the levels of ferritin, lactate dehydrogenase, urea, creatinine, BUN, total bilirubin, direct bilirubin, indirect bilirubin, AG ratio, alanine transaminase and gamma glutamyl transferase and significant decrease in the levels of chloride, total protein, albumin in severe positive (ferritin levels >500ng/ml) compared to mild positive (ferritin levels <500ng/ml) [Table 4].

A significant positive correlation of ferritin was found with lactate dehydrogenase, urea, creatinine, BUN, potassium, total bilirubin, direct bilirubin, indirect bilirubin, aspartate transaminase, alanine transaminase and alkaline phosphatase. A significant negative correlation of ferritin was found with chloride and albumin in COVID positive patients [Table 5]. A significant positive correlation of ferritin was found with lactate dehydrogenase, bicarbonate and gamma glutamyl transferase in mild positive patients [Table 5]. A significant positive correlation of ferritin was found with lactate dehydrogenase, urea, creatinine, BUN, potassium, total bilirubin, direct bilirubin, aspartate transaminase, alanine transaminase and alkaline phosphatase. A significant negative correlation of ferritin was found with chloride in severe positive patients [Table 5].

We observed the frequency of participants in mild positive as 84 (31%), severe positive as 65 (24%) and negative as 121 (45%). We also observed a significant difference in

the levels of urea, creatinine, BUN, sodium, total bilirubin, indirect bilirubin, total protein, aspartate transaminase, alanine transaminase and alkaline phosphatase between mild positive, severe positive and negative groups in ANOVA analysis [Tables 6, 7, 8].

Table 5. Pearson Correlation Of Ferritin With Study Parameters In Positive Group, Mild Positive And Severe Positive Groups

Ferritin association with study parameters	Positive group			Mild positive			Severe positive		
	N	R value	P-value	N value	R value	P-value	N	R value	P-
LDH (U/L)	110	0.562	0.000***	69	0.266	0.027*	41	0.495	0.001**
Urea (mg/dl)	147	0.590	0.000***	84	-0.034	0.762	63	0.577	0.000***
Creatinine(mg/dl)	149	0.575	0.000***	84	0.055	0.619	65	0.694	0.000***
BUN (mg/dl)	149	0.589	0.000***	84	0.760	-0.034	65	0.581	0.000***
Sodium (mmol/l)	146	-0.090	0.281	82	0.060	0.591	64	-0.035	0.782
Potassium (mmol/l)	145	0.167	0.045*	82	-0.105	0.350	63	0.275	0.029*
Chloride (mmol/l)	145	-0.305	0.000***	82	-0.116	0.300	63	-0.312	0.013*
Bicarbonate (mmol/l)	145	-0.105	0.208	82	0.233	0.035*	63	-0.170	0.182
Total bilirubin (mg/dl)	149	0.260	0.001**	84	0.193	0.079	65	0.256	0.039*
Direct bilirubin (mg/dl)	149	0.272	0.001**	84	0.167	0.128	65	0.270	0.030*
Indirect bilirubin (mg/dl)	149	0.163	0.047*	84	0.137	0.215	65	0.167	0.185
Total protein (g/dl)	149	-0.146	0.075	84	-0.043	0.697	65	-0.116	0.356
Albumin (g/dl)	149	-0.199	0.015*	84	0.031	0.782	65	-0.143	0.257
Globulin (g/dl)	149	-0.072	0.382	84	-0.118	0.286	65	-0.033	0.794
AG ratio	149	-0.131	0.112	84	0.136	0.217	65	-0.115	0.361
AST (IU/L)	149	0.440	0.000***	84	-0.077	0.489	65	0.438	0.000***
ALT (IU/L)	148	0.410	0.000***	83	0.064	0.565	65	0.374	0.002**
ALP (IU/L)	148	0.334	0.000***	83	0.054	0.630	65	0.330	0.007**
GGT (U/L)	149	0.112	0.174	84	0.385	0.000***	65	-0.030	0.811

Significance: *P<0.05, **P<0.01, ***P<0.001

We observed that the prevalence of COVID infection was more in males compared to females. Similar results were observed by Huang et al. (2020) and Chen et al. (2020). MERS-CoV and SARS-CoV have also been found to infect more males than females (Badawi et al. 2016). These findings could be linked to the protection associated with sex hormones which modulate both innate and adaptive immunity (Jaillon et al. 2019; Channappanavar et al. 2020). The mean age of COVID positive group was found to be 50.28 ± 15.19 years in our study. We observed that a greater number of COVID positive patients were in the age group of more than 60 years (30.2%) whereas an increased incidence of COVID infection in the age group of 50-59years (30%) has been reported (Chen et al. 2020).

Ferritin is an iron-binding molecule which helps to store iron in its active form and protects from iron toxicity. Each apoferritin is made up of 24 subunits of two types namely the H and L subunits. The L-subunit rich ferritin predominantly occurs in liver, spleen whereas the H-subunit rich ferritin is present in heart and kidneys (Harrison et al. 1996; Knovich et al. 2009). Oxidative stress, growth factors, thyroid hormone, second messengers, hyperoxia and hypoxia-ischemia are some of the major factors regulating the expression of ferritin (Torti et al. 2002). The hepatocytes, kupffer cells and macrophages secrete ferritin (Recalcati et al. 2008; Wang et al. 2010; Cohen et al. 2010). The serum ferritin (iron poor form) is mostly made up of L-subunits only. H-ferritin with its immunomodulatory effects causes diminished antibody production, allergic responses as well as phagocytosis (Broxmeyer et al. 1981; Hann et al. 1989; Morikawa et al. 1994; Recalcati et al. 2008). The L-subunit (predominant in serum) acts as a pro-inflammatory mediator (Ruddell et al. 2009; Wang et al. 2010; Channappanavar et al. 2020).

Ferritin expression is found to be induced by pro-inflammatory cytokines (IL-6) and in turn ferritin enhances the expression of the pro-inflammatory cytokines. Moreover, induction of the anti-inflammatory cytokines (IL-10) also by ferritin explains its immunosuppressive effects. Ferritin as well as the cytokines regulate both inflammation and immunosuppression. Hence ferritin can play a role as either an immunosuppressive or a pro-inflammatory agent through different receptors, pathways and effectors (L- versus H-ferritin). An already existing proinflammatory state, sepsis or genetic susceptibility may influence the pathogenic role of ferritin (Rosário et al. 2013). Several mechanisms have been suggested to explain the association

of hyperferritinemia with severity of disease in COVID-19 patients. The viral infection mediated production of the proinflammatory cytokines (IL-l β, IL-6, TNF- α) as well as leakage of intracellular ferritin as a consequence of inflammation mediated cellular damage have been implicated as the cause of hyperferritinemia commonly occurring in COVID-19 patients. Heightened reactive oxygen species driven iron release from ferritin may also promote a vicious cycle of inflammation (Kobune et al. 1994; Kell et al. 2014; Channappanavar et al. 2020).

Table 6. Multiple Comparison Of Study Parameters In Mild Positive, Severe Positive And Negative Groups By Anova

Study parameters		Sum of Squares	df	Mean Square	F	Sig.
LDH	Between Groups	11560407.152	249	46427.338	1.832	.056
	With in Groups	506958.167	20	25347.908		
	Total	12067365.319	269	1252.390	2.667	.007**
Urea	Between Groups	310592.609	248			
	With in Groups	8921.167	19	469.535		
	Total	319513.776	267			
Creatinine	Between Groups	1054.846	249	4.236	50.233	.000***
	With in Groups	1.687	20	.084		
	Total	1056.533	269			
BUN	Between Groups	68319.249	249	274.374	2.815	.004**
	With in Groups	1949.185	20	97.459		
	Total	70268.434	269			
Sodium	Between Groups	22278.939	242	92.062	7.498	.000***
	With in Groups	221.000	18	12.278		
	Total	22499.939	260			
Potassium	Between Groups	67.761	241	.281	1.338	.240
	With in Groups	3.782	18	.210		
	Total	71.542	259			
Chloride	Between Groups	4503.938	241	18.689	1.214	328
	With in Groups	277.000	18	15.389		
	Total	4780.938	259			
Bicarbonate	Between Groups	5635.395	241	23.383	.155	1.000
	With in Groups	2717.667	18	150.981		
	Total	8353.062	259			

Significance: *P<0.05, **P<0.01, ***P<0.001

Table 7. Multiple Comparison Of Study Parameters In Mild Positive, Severe Positive And Negative Groups By Anova

Study parameters		Sum of Squares	df	Mean Square	F	Sig.
Total bilirubin	Between Groups	36.355	248	.147	2.253	. .018*
	With in Groups	1.301	20	.065		
	Total	37.657	268			
Direct bilirubin	Between Groups	9.959	248	.040	1.653	.092
	With in Groups	.486	20	.024		
	Total	10.445	268			
Indirect bilirubin	Between Groups	19.426	248	.078	2.380	.013*
	With in Groups	.658	20	.033		
	Total	20.084	268			
Total protein	Between Groups	188.533	248	.760	2.553	.008**
	With in Groups	5.955	20	.298		
	Total	194.488	268			
Albumin	Between Groups	106.440	248	.429	1.536	.129
	With in Groups	5.590	20	.280		
	Total	112.030	268			
Globulin	Between Groups	49.981	248	.202	1.559	.121
	With in Groups	2.585	20	.129		
	Total	52.566	268			
AG ratio	Between Groups	16.718	249	.067	1.416	.181
	With in Groups	.948	20	.047		
	Total	17.667	269			

Significance: *P<0.05, **P<0.01, ***P<0.001

Based on the ferritin values obtained, the patients were categorized into mild and severe groups using a cut-off value of 500 ng/ml. Among the 149 positive cases, 84 (56.4%) were found to have ferritin levels <500ng/ml and 65 (43.6%) had ferritin levels >500 ng/ml whereas of the 121 negative cases, 107 (88.4%) had ferritin level <500 ng/ml and 14 (11.6%) had ferritin levels >500 ng/ml. Serum ferritin levels are also found to be increased in a variety of diseases and other inflammatory states also especially diabetes mellitus which could explain this finding (Lin et al. 2020). Analysis of 147 confirmed cases of COVID-19 found 29.93 % of patients to have hyperferritinemia (>500 ng/ml) whereas in our study 43.6% of patients had hyperferritinemia (Lin et al. 2020).

We reported significant increase in serum ferritin levels in severe positive samples (1449.84 ± 249.47) compared to mild positive samples (230.04 ± 17.41). Zhou et al found that individuals with severe COVID infection exhibited greater elevations of serum ferritin levels (Zhou et al. 2020). Jenifer et al in their review of studies which documented serum ferritin levels among severe and non-severe COVID-19 patients only at the time of hospital admission observed ferritin concentrations to be within the normal range in non-severe disease and presence of hyperferritinemia in severe disease state (Gomez-Pastoraa et al. 2020). These findings are similar to the observations of our study. Bilateral pulmonary infiltration rate has been observed to be more in patients who had ferritin levels more than 500 ng/

Aravaanan et al.,ml (Lin et al.2020). On investing 201 COVID- 19 patients they found increased risk of development of ARDS to be associated with higher serum ferritin levels (Wu et al.2020). On the other hand, they found ferritin levels to be elevated in both mild as well as in severe. Among the non survivors, ferritin levels remained elevated throughout the course of the disease (Mo et al. 2020; Zhou et al. 2020).

Table 8. Multiple Comparison Of Study Parameters In Mild Positive, Severe Positive And Negative Groups By Anova

Study parameters		Sum of Squares	df	Mean Square	F	Sig.
Aspartate Groups	Between transaminase	3103587.502	248	12514.466	90.450	.000***
	With in Groups	2767.167	20	138.358		
	Total	3106354.669	268			
Alanine Groups	Between transaminase	1256918.527	247	5088.739	16.166	.000***
	With in Groups	6295.667	20			
	Total	1263214.194	267	314.783		
Alkaline Groups	Between phosphatase	1131998.188	247	4582.989	2.108	.026*
	With in Groups	43489.167	20			
	Total	1175487.354	267	2174.458		
Gamma Groups transferase	Between glutamyl	518302.004	248	2089.927	. 922	.634
	With in Groups	45353.000	20			
	Total	563655.004	268	2267.650		

Significance: *P<0.05, **P<0.01, ***P<0.001

Thus, ferritin levels estimated at admission as well as during the progress of the disease may help to differentiate those with severe manifestations and help in planning effective treatment strategies. Circulating ferritin levels may not only indicate an acute phase response but may also play a key inflammatory role in pathogenesis of COVID-19. Hence the role of iron chelators as well as reduction of dietary iron can also be considered as treatment strategies in the setting of hyperferritinemia (Fleming et al. 2002; Mobarra et al. 2016). LDH, an intracellular enzyme which occurs in cells of most organs is composed of two major subunits and occurs as five major isozymes. Cytokine mediated tissue damage following severe infection causes LDH release into circulation (Martinez-Outschoorn et al. 2011; Ju et al. 2016; Henry et al. 2020).

The isoenzyme of LDH derived from the lungs (LDH-3) is found to be elevated as a consequence of interstitial pneumonia which may progress on to acute respiratory distress syndrome, a common feature of COVID (Kaplan et al. 2002; Patschan et al. 2006; Zhang et al. 2014). Early laboratory data analysis of COVID-19 patients had indicated significant variations in LDH levels among patients with severe disease manifestations (Henry et al. 2020). We observed that 58.2% of COVID positive patients had LDH levels greater than 280 U/L. By January 2020, 73% of COVID infected patients with elevated LDH levels was reported (Huang et al.2020). LDH levels were also significantly elevated in the severe positive group (ferritin >500ng/ml). A meta-analysis of 9 published studies with 1532 COVID-19 patients to study the association between elevated LDH levels at the time of admission and disease outcome and found that elevated LDH levels represented a sixfold increase in the risk of developing severe disease and a sixteen-fold increase in mortality (Henry et al. 2020).

Greater increase in LDH levels was seen among patients admitted in the intensive care unit and those who progressed onto acute respiratory distress syndrome (ARDS) respectively (Huang et al 2020; Wu et al. 2020). Nearly 60 % of patients with SARS and also those infected with MERS-CoV have reported liver impairment (Chau et al. 2004; Alsaad et al. 2018). Direct viral infection of the hepatic cells has been implicated as the cause for liver damage in patients infected with coronavirus. Data from large scale case studies have indicated that 2-11% of COVID patients had pre-existing liver comorbidities, 14-53% had alterations in levels of AST and ALT as the disease progressed. Liver dysfunction is found to be more prevalent in severe than in mild cases of COVID (Zhang et al. 2020).

In our study elevations of total bilirubin occurred in 12.7%, AST in 65.8 %, ALT in 44.3 %, ALP in 9.4% and GGT in 51.7% of COVID positive patients. Hypoproteinemia and hypoalbuminaemia was observed in 23.5% of the patients. An increase in ALT by 28%, AST by 35%, bilirubin by 18% and decrease in albumin by 98% and elevation of GGT by 54% was reported (Chen et al. 2020; Zhang et al. 2020).

Table 9. Incidence Of Liver Function Parameters

Parameters ranges	Positive participants	Negative participants
Total bilirubin (mg/dl)	Positive (n-149)	Negative (n-120)
<0.5	53 (35.6%)	55 (45.8%)
0.5-1.0	77 (51.7%)	55 (45.8%)
>1.0	19 (12.7%)	10 (8.4%)
Direct bilirubin (mg/dl)	Positive (n-149)	Negative (n-120)
<0.1	26 (17.4%)	44 (36.7%)
0.1-0.3	98 (65.8%)	67 (55.8%)
>0.3	25 (16.8%)	9 (7.5%)
Indirect bilirubin (mg/dl)	Positive (n-149)	Negative (n-120)
<0.2	11 (7.4%)	4 (3.4%)
0.2-0.8	125 (83.9%)	109 (90.8%)
>0.8	13 (8.7%)	7 (5.8%)
Total Protein (g/dL)	Positive (n-149)	Negative (n-120)
<6.6	35 (23.5%)	14 (11.7%)
6.6-8.3	110 (73.8%)	105 (87.5%)
>8.3	4 (2.7%)	1 (0.8%)
Albumin (g/dL)	Positive (n-149)	Negative (1-120)
<3.5	35 (23.5%)	18 (15%)
3.5-5.2	113 (75.8%)	102 (85%)
>5.2	1 (0.7%)	0 (0%)
Globulin (g/dL)	Positive (n-149)	Negative (n-120)
<2.5	7 (4.7%)	1 (0.8%)
2.5-3.0	45 (30.2%)	38 (31.7%)
>3.0	97 (65.1%)	81 (67.5%)
AG ratio	Positive (n-149)	Negative (n-120)
<1.4	120 (80.6%)	86 (71.7%)
1.4-1.7	23 (15.4%)	31 (25.8%)
>1.7	6 (4.0%)	3 (2.5%)
AST (IU/L)	Positive (n-149)	Negative (n-120)
<31	51 (34.2%)	62 (51.7%)
>31	98 (65.8%)	58 (48.3%)
ALT (IU/L)	Positive (n-149)	Negative (n-120)
<34	83 (55.7%)	83 (69.2%)
>34	66 (44.3%)	37 (30.8%)
ALP (IU/L)	Positive (n-149)	Negative (n-120)
<30	2 (1.3%)	0 (0%)
30-120	133 (89.3%)	111 (92.5%)
>120	14 (9.4%)	9 (7.5%)
GGT (U/L)	Positive (n-149)	Negative (n-120)
<38	72 (48.3%)	79 (65.8%)
>38	77 (51.7%)	41 (34.2%)

Inflammatory cytokines are known to play a role in inducing acute kidney injury and glomerulopathy. Endothelial injury and cardiovascular instability occurring in severely infected COVID patients may cause renal impairment resulting in cardio renal syndrome (Gonza´lez-Cuadrado et al. 1997; Sanz et al. 2011; Lin et al. 2020; Fan et al. 2020). Previous studies have reported expression of angiotensin converting enzyme 2 (ACE-II) receptors in human kidneys thus indicating a potential pathway for COVID-19 infection (Lin et al. 2020; Fan et al. 2020). Acute kidney injury

is considered to be an independent predictor of covid-19 in-hospital mortality (Cheng et al. 2020; Carriazo et al. 2020). Analysis of urea and creatinine levels at the onset of disease as well as later during the clinical course showed that impaired kidney function is seen in COVID-19 patients contributing to morbidity and mortality (Hassan Mohammed et al; Mahmoudi et al. 2020).

Table 10. Incidence Of Kidney Function Parameters

Parameters ranges	Positive participants	Negative participants
Urea (mg/dL)	Positive (n-147)	Negative (n-121)
<17	15 (10.2%)	31 (25.6%)
17-43	105 (71.4%)	84 (69.4%)
>43	27 (18.4%)	6 (5.0%)
Creatinine (mg/dL)	Positive (n-147)	Negative (n-121)
<0.5	2 (1.4%)	8 (6.6%)
0.5-1.2	124 (84.3%)	109 (90.1%)
>1.2	21 (14.3%)	4 (3.3%)
BUN (mg/dL)	Positive (n-147)	Negative (n-121)
<6	4 (2.7%)	8 (6.6%)
6-20	116 (78.9%)	107 (88.4%)
>20	27 (18.4%)	6 (5.0%)

Table 11. Incidence Of Electrolyte Imbalances

Parameters ranges	Positive participants	Negative participants
Sodium (mmol/L)	Positive (n-145)	Negative (n-115)
<130	11 (7.6%)	1 (0.9%)
130-145	132 (91%)	113 (98.2)
>145	2 (1.4%)	1 (0.9)
Potassium (mmol/L)	Positive (n-145)	Negative (n-115)
<3.5	14 (9.7%)	7(6.1%)
3.5-5.0	121 (83.4%)	107 (93%)
>5.0	10 (6.9%)	1 (0.9%)
Chloride (mmol/L)	Positive (n-145)	Negative (n-115)
<95	14 (9.7%)	1 (0.9%)
95-105	116 (80%)	96 (83.5%)
>105	15 (10.3%)	18 (15.6%)
Bicarbonate (mmol/L)	Positive (n-145)	Negative (n-115)
<21	28 (19.3%)	7(6.1%)
21-31	114 (78.6%)	107 (93%)
>31	3 (2.1%)	1 (0.9%)

In our study, elevations of urea levels occurred in 18.4%, creatinine in 14.3% and BUN in 18.4% was found in COVID positive patients. Creatinine elevations have been reported as 10 % and 3% in their COVID study group by Huang et al. (2020); Chen et al. (2020) respectively. An increase in BUN beyond the normal range was seen in 6% of the study group analysed by Chen et al. The meta-analysis on biochemical abnormalities also identified derangements in kidney function in patients with severe and fatal COVID-19 patients (Henry et al. 2020). Initial evidence from COVID studies have indicated the presence of electrolyte abnormalities (Guan et al. 2020; Huang et al. 2020). Identification of such alterations help not only in effective patient management but also help to understand key pathophysiological mechanisms of the disease process. Among the COVID patients of our study, the incidence of hyponatremia and hypokalemia was found to be 7.6% and 9.7% respectively. Fall in serum chloride and bicarbonate levels below the reference range was noted in 9.7% and 19.3% respectively (Lippi et al. 2020). The abnormalities in serum electrolyte levels were found to be more prevalent among the positive patients compared to those who had tested negative. Results of pooled analysis identifies the most commonly encountered electrolyte abnormalities in COVID patients which indicates the COVID-19 severity to

be associated with lowered serum concentrations of sodium, potassium and calcium (Lippe et al. 2020).

Binding of the virus to its host receptor namely angiotensin converting enzyme-2 may cause increased renal loss of potassium leading onto hypokalemia. Electrolyte status among COVID patients also varies highly (Lippe et al. 2020; Huang et al. 2020). Studies with larger number of COVID positive samples analysed at different stages of progression of the disease will help to clearly establish the clinical significance and to initiate the appropriate interventions. The major limitation of our study is its retrospective cross-sectional nature and the non-availability of correlation with the clinical aspects of COVID patients. Analysis of a larger sample size sourced from multiple centres with clinical outcome can help in a better understanding of the clinical utility of these parameters.

CONCLUSION

The findings of the present study indicate that males were more commonly affected by COVID-19 than females. The maximum numbers of positive cases were found in the age group of more than 60 years. Among the 149 positive cases, 84 (56.4%) were found to have ferritin levels <500 ng/ml and 65 (43.6%) had ferritin levels >500 ng/ml. We reported significant increase in serum ferritin levels in severe positive samples (1449.84 ± 249.47) compared to mild positive samples (230.04 ± 17.41). We observed significant increase in the levels of lactate dehydrogenase, urea, creatinine, BUN, total bilirubin, direct bilirubin, indirect bilirubin, AG ratio, alanine transaminase and gamma glutamyl transferase and significant decrease in the levels of chloride, total protein, albumin in severe positive (ferritin levels >500 ng/ml) compared to mild positive (ferritin levels <500 ng/ml). The significant alterations in various biochemical parameters among the COVID-19 patients suggests the importance of initial assessment and monitoring of these laboratory parameters in risk assessment.

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