Original Research Article

Biochemical alterations in covid-19 patients during the decline of first wave of the pandemic

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Abstract

Background: "COVID-19 evokes coagulation dysfunctions exhibited with elevated serum LDH and D-Dimer levels."**Aim:** The aim of this study was to evaluate the circulating biochemicals in confirmed COVID-19 patients on 1st, 7th and 14th day of hospitalization, their association with disease severity and correlate the values with various organ functions based on metabolic functions. **Materials and Methods:** A total of 483, RT-PCR confirmed COVID-19 patients were ordered for a panel of blood biochemistry tests and inflammatory markers such as blood LDH, D-Dimer, LFT and RFT on 1st, 7th and 14th day of hospitalization. Regression analysis was adjusted by relevant factors and confounding variables for all concerned electronically collected medical data. **Results:** Determination of coagulation factors such as serum LDH and D-Dimer in hospitalised COVID-19 patients evidenced a consistent association during patient's hospital stay, prognosis of the disease and with recovery rates. A similar trend was found for other biochemical variables. Serum total protein and hypoalbuminemia proved to correlate negative with coronavirus infection with AGR improving with hospital stay. **Conclusion:** LDH along with coagulation factor D-Dimer and other organ-specific biomarkers accompanied good clinical and metabolic outcomes in mild to moderate COVID-19-infected patients. Levels of LDH and D-Dimer may be suitable enough individually to categorize patients as mild, moderate and severe to manage the present pandemic.

Clinical significance: The laboratory values of LDH and D-Dimer may enhance early knowledge of the clinician about the severity of COVID-19, prognosis of the disease, treatment regimen.

Key words: COVID-19, coronavirus, biochemical alterations, LDH, D-Dimer, laboratory research

Introduction

The outbreak of COVID-19, a novel, highly transmittable viral infection, similar to the Middle East respiratory syndrome coronavirus (MERS-Co V) and severe acute respiratory syndrome coronavirus (SARS-Co V), was reported in the human population at the end of December 2019 in Wuhan, Hubei province of central China and now the so called 'smart', 'unstoppable' virus has succeeded in spreading across the whole planet with tremendous rate of fatality [1]. WHO named it 'COVID-19' and declared it a pandemic in March 2020. As per current records from WHO, the number of COVID-19 cases globally have exceeded 197 million by 30th July 2021 and in a small Indian peninsular country, the total confirmed cases rose to 31.6 million [2].

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The clinical presentation of coronavirus disease ranges from asymptomatic to mild to moderate to critically ill condition. In most COVID-19 cases, patients experience mild influenza type of illness with no symptoms; a moderate number of patients suffer severe pneumonia, acute respiratory distress syndrome (ARDS), multiple organ failure (MOF) and some succumb to even death [3,4]. Thus, creating hurdle for clinicians in early recognition of COVID-19 subjects who tend to develop severe infection later.

The elderly people with preexisting co-morbidities such as diabetes, Cardio Vascular Disease (CVD), Chronic Respiratory Disease, Chronic Kidney Disease (CKD) when infected with coronavirus disease might have serious consequences [5]. Some scientific studies have reported several biochemical abnormalities in association with SARS-CoV-2 infection, its severity and some parameters are reported to be considered specific disease biomarkers [6]. Biochemical investigations are worth being performed to predict severity, co-morbidity and better follow-up of COVID-19 patients [7, 8]. Thus, early diagnosis, identification and isolation of positive cases and with apt treatment protocol, the existing COVID-19 pandemic can be tackled [9, 10]. Various studies have reported an average incubation period for COVID-19 between 6 and 7 days and the first viremic phase lasting 8–10 days, followed by infection resolution [11]. We decided to conduct a pilot retrospective study to assess whether there were significant differences in the biochemical patterns in

hospitalized COVID-19 patients between 1st and 14th day, evaluate their association with disease severity and risk to various organs.

Materials and methods

Study design and participants recruited

This study is a single-centre retrospective cohort study conducted on 483 confirmed COVID-19 patients between September and November 2020, at Parul Sevashram Hospital (PSH), a tertiary care Hospital in Vadodara, designated by Gujarat Government as a hospital for treating COVID-19 patients. Data on different laboratory parameters were collected at the admission (i.e., Day 1), a week after (Day 7), and two weeks after (Day 14).Patient's data were collected from the Medical Records Department (MRD) of the Hospital. Laboratory real-time reverse-transcriptase–polymerase-chain-reaction (RT-PCR) [4] was performed to confirm COVID-19. The Ethical Clearance was taken from the Institutional Ethics Committee (PUIECHR/PIMSR/00/081734/2901) and de- identified secondary data was used for this analysis after ethical approval.

Criteria of selection

Inclusion criteria were RT-PCR confirmed, COVID-19 patients, both males and females between 18 and 80 years of age, who reported to the hospital. Those excluded from the study were non-confirmed COVID-19 patients, below 18 and above 80 years. Patient's identification number and records of data collection were stored in a confidential environment.

Laboratory tests of study variables

A standardized protocol was followed for collecting patient's blood sample for routine laboratory testing at PSH. Routine biochemical tests were performed on patients' admission to the isolation ward/ICU of the hospital as per IFCC recommendations.

Statistical analysis

For descriptive presentation of all laboratory parameters, mean, standard deviation (SD), median, and inter quartile range (IQR) was used, and frequency with proportions for categorical variables. Observed laboratory parameters were compared using Mann–Whitney U test between gender, and using Kruskal-Wallis H test between age groups. Further, exploratory linear mixed-effect models were used to explore the average change in each laboratory parameter between day 1 and day 14 by adjusting age and gender. Estimated average change from day 1 with 95% confidence interval (CI) were reported throughout in this manuscript. Interaction between age and gender with time was also explored and reported if necessary (considered significant if $p < 0.1$).All p-values were based on two-tailed tests of significance and those < 0.05 was deemed statistically significant. All data were entered and stored in MS Excel, and analysed using Stata 16.1 (StataCorp, College Station, Taxas, USA) statistical analysis software.

Result

The study population included 483 confirmed COVID-19 patients visiting the emergency department of a tertiary care hospital. The mean age was 51.6 years (SD: 15.3), and the majority were male (n=320, 66.3%) and females (n=163, 33.7%). Further, nearly half of them (n=229, 47.4%) were in the age range of 40–60 years followed by more than 60 years (n=148, 30.6%), and <40 years (n=106, 22.0%) respectively

Table 1: Distribution of biochemical parameters on day one (admission), day seven, and day fourteen

Biochemical parameters	Day 1					Day 7				Day 14			
	N	Mean (SD)	Median	IQR		N	Mean (SD)	Median	IQR	N	Mean (SD)	Median	IQR
LDH (U/L)	373	606.3 (678.6)	505.4	377 - 685.4		45	506.4 (298.3)	459.0	289 - 637.5	13	466.9 (259.5)	413.0	244.0 - 642
D-D (ng/ml)	366	693.7 (1706.7)	178.0	104.2 - 383.1		-	-	-	-	-	-	-	-
TB (mg/dl)	469	0.6 (0.4)	0.5	0.4 - 0.6		100	0.7 (0.7)	0.5	0.4 - 0.8	50	0.6 (0.3)	0.5	0.4 - 0.6
DB (mg/dl)	468	0.3 (0.2)	0.2	0.2 - 0.3		100	0.4 (0.4)	0.2	0.2 - 0.4	50	0.3 (0.2)	0.2	0.2 - 0.3
IB (mg/dl)	469	0.4 (2.2)	0.3	0.2 - 0.4		100	0.4 (0.3)	0.3	0.2 - 0.4	50	0.3 (0.2)	0.3	0.2 - 0.4
PT (U/L)	474	31.8 (34.2)	21.0	17.0 - 35.0		100	38.0 (29.8)	26.5	18 - 52.5	49	36.5 (24.4)	30.0	19.0 - 44.0
OT (U/L)	469	35.0 (59.0)	24.0	18.0 - 36.0		100	30.4 (24.4)	23.0	17.0 - 36.0	50	25.3 (13.3)	20.0	15.0 - 28.0
ALP (U/L)	469	66.1 (45.1)	57.0	45.0 - 79.0		102	59.8 (24.6)	56.0	42.0 - 70.0	51	75.4 (33.0)	68.0	50.0 - 96.0
TP (g/dl)	469	6.8 (5.5)	6.2	5.8 - 6.6		102	5.5 (0.7)	5.5	5.0 - 6.0	51	5.3 (0.7)	5.4	4.9 - 5.9
A (g/dl)	468	3.4 (1.5)	3.4	3.0 - 3.6		102	2.9 (0.5)	2.8	2.6 - 3.3	51	2.7 (0.5)	2.7	2.4 - 3.0
G (g/dl)	468	2.8 (0.7)	2.8	2.4 - 3.4		103	2.6 (0.6)	2.5	2.3 - 3.0	51	2.7 (0.5)	2.7	2.3 - 3.0
A:G	468	1.5 (2.0)	1.2	0.9 - 1.5		102	1.1 (0.4)	1.1	0.9 - 1.3	51	1.0 (0.3)	1.0	0.8 - 1.2
Ur (mg/dl)	460	31.5 (21.7)	26.0	19.0 - 36.0		98	44.2 (32.9)	34.0	26.3 - 46.3	46	42.4 (31.9)	32.0	22.8 - 50.0
Cr (mg/dl)	474	1.1 (1.2)	0.8	0.7 - 1.0		108	1.1 (1.1)	0.8	0.7 - 1.0	51	1.2 (1.2)	0.8	0.7 - 1.1
UA (mg/dl)	457	5.4 (8.0)	4.4	3.3 - 5.7		97	5.0 (9.7)	3.6	2.7 - 4.9	47	6.1 (14.9)	3.2	2.2 - 4.1
Na+(mmol/L)	375	137.1 (14.1)	139.0	136.0 - 142.0		63	137.1 (18.1)	139.0	137 - 141	35	140.5 (7.5)	139.0	136.0 - 141.0

K+(mEq/L)	376	4.7 (5.3)	4.3	4.0 - 4.7		62	4.4 (0.6)	4.3	4.0 - 4.7	37	4.3 (0.5)	4.1	3.8 - 4.5
Cl-(mmol/L)	373	104.0 (10.5)	106.0	102.0 - 108.0		44	105.4 (5.0)	106.0	102.0 - 109.0	34	104.2 (7.2)	102.5	100.0 - 106.0

SD: Standard Deviation; IQR: Interquartile Range; LDH: Lactate Dehydrogenase; DD: D-Dimer; TB: Total Bilirubin; DB: Direct Bilirubin; IB: Indirect Bilirubin; SGPT: Serum Glutamate Pyruvate Transaminase; SGOT: Serum Glutamate Oxaloacetate Transaminase; ALP: Alkaline Phosphatase; TP: Total Protein; A: Albumin; G: Globulins; A:G: Albumin:Globulin; Ur: Urea; Cr: Creatinine; UA: Uric Acid; Na+: Sodium; K+: Potassium; Cl-: Chloride

[Table 1] shows the descriptive distribution for laboratory parameters of COVID-19 confirmed hospitalized patients from 1st day through 7th to 14th day. Serum Lactate Dehydrogenase was markedly elevated in COVID-19 patients, on the first day, with a mean of 606.3 U/L (SD=678.6) and gradually decreased to 506.4 U/L (SD=298.3) on 7th day followed by 466.9 U/L (SD=259.5) on day 14. The D-Dimer was reported to be raised with a mean of 693.7ng/ml (SD=1706.7) on the first day of admission. Mean serum Total Proteins on the first day were in the normal range; 6.8 gm/dl (SD=5.5) with a subsequent decrease on 7th and 14th day of hospitalization. Similarly, serum albumin and Globulins also showed a decline in the levels from 1st to 14th day. Albumin:Globulin (A:G) ratio was initially more but returned to normal by 14th day. Though serum total bilirubin levels were within normal limits, and direct Bilirubin was slightly elevated on all three study days. Liver enzymes serum glutamate Pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT) and Alkaline phosphatase (ALP) were well within the reference range on all three specified days. Blood urea levels were in the normal range but toward higher end 44.2mg/dl (SD=32.9) on 7th day of hospitalization.

Table 2: Comparison of biochemical parameters by gender and age groups on day one

Biochemical parameters	Male				Female			P-value[1]	Age <40 years				Age 40–60 years				Age >60 years			P-value[2]
	N	Mean	SD		N	Mean	SD		N	Mean	SD		N	Mean	SD		N	Mean	SD	
LDH (U/L)	251	636.7	671.2		122	543.9	304.1	0.121	81	470.5	260.2		178	649.6	748.0		114	635.2	400.8	p<0.001
D-D (ng/ml)	236	639.1	1615.2		130	792.7	1863.8	0.479	83	509.6	1377.9		179	665.0	1774.1		104	889.8	1817.6	p<0.001
TB (mg/dl)	309	0.7	0.5		160	0.4	0.2	p<0.001	103	0.5	0.3		222	0.6	0.5		144	0.6	0.4	0.219
DB (mg/dl)	308	0.3	0.2		160	0.2	0.1	p<0.001	103	0.2	0.1		222	0.3	0.2		143	0.3	0.2	0.281
IB (mg/dl)	309	0.5	2.7		160	0.3	0.2	p<0.001	103	0.8	4.6		222	0.3	0.4		144	0.3	0.2	0.219
PT (U/L)	313	32.7	26.1		161	30.1	46.1	0.005	105	27.3	20.0		224	37.9	44.7		145	25.7	18.8	p<0.001
OT (U/L)	309	34.1	33.8		160	36.7	89.6	0.003	103	24.5	13.8		222	41.8	82.6		144	32.0	23.4	p<0.001
ALP (U/L)	309	67.3	52.2		160	63.6	25.7	0.635	103	61.2	26.9		222	70.8	58.5		144	62.3	27.3	0.243
TP (g/dl)	309	6.3	2.1		160	7.8	8.9	0.053	103	6.3	0.8		222	6.7	5.2		144	7.3	7.5	0.040
A (g/dl)	308	3.4	0.6		160	3.5	2.4	0.741	103	3.7	0.7		221	3.3	0.4		144	3.4	2.5	p<0.001
G (g/dl)	308	2.9	0.7		160	4.3	0.7	0.111	103	2.6	0.8		221	3.4	0.7		144	3.9	0.7	0.099
A:G	308	1.17	2.4		160	0.81	0.5	0.166	103	1.4	1.4		221	0.97	2.2		144	0.87	2.0	p<0.001
Ur (mg/dl)	304	33.6	23.8		156	27.3	16.1	p<0.001	100	24.5	14.1		219	29.9	23.3		141	38.8	21.6	p<0.001
Cr (mg/dl)	314	1.1	1.1		160	1.0	1.3	p<0.001	103	1.1	1.4		224	1.0	1.0		147	1.2	1.2	p<0.001
UA (mg/dl)	301	5.9	9.7		156	4.4	1.9	0.005	99	5.3	7.5		218	5.5	9.8		140	5.1	4.4	0.743
Na+(mmol/L)	249	136.4	15.1		126	138.6	11.7	0.002	85	140.6	2.8		179	135.4	19.7		111	137.4	5.3	p<0.001
K+(mEq/L)	250	4.9	6.4		126	4.3	0.6	0.186	85	4.3	0.5		180	5.1	7.6		111	4.4	0.6	0.376
Cl-(mmol/L)	247	103.7	8.4		126	104.6	13.7	p<0.001	84	104.9	12.0		178	104.1	9.3		111	103.2	11.1	0.009

[1]P-values were estimated using Mann–Whitney U test; [2]P-values were estimated using the Kruskal-Wallis nonparametric analysis of variance; LDH: Lactate Dehydrogenase; DD: D-Dimer; TB: Total Bilirubin; DB: Direct Bilirubin; IB: Indirect Bilirubin; SGPT: Serum Glutamate Pyruvate Transaminase; SGOT: Serum Glutamate Oxaloacetate Transaminase; ALP: Alkaline Phosphatase; TP: Total Protein; A: Albumin; G: Globulins; A:G: Albumin:Globulin; Ur: Urea; Cr: Creatinine; UA: Uric Acid; Na+: Sodium; K+: Potassium; Cl-: Chloride

Table 2 reflects the first day's comparison of biochemical variables by gender and age groups. Most of the variables were within the normal range. Mean LDH (U/L) values were high in both male and female patients (p=0.121). Serum LDH was high in all three age group subjects with the highest values 649.6 IU/L (SD=748.0) being recorded in, 40-60-year-old patients, followed by 635.2 IU/L (SD=400.8) in more than 60 years and 470.5 IU/L (SD=260.2) in <40 years old patients with a statistically significant p<0.001. The mean inflammatory marker D-Dimer was much above the normal reference range in both genders (females: 792.7ng/ml (SD=1863.8) and males: 639.1ng/ml (SD=1615.2), p=0.479). However, elderly patients (>60 years) showed significantly higher (p<0.001) mean D-Dimer scores compared to other age groups. The liver enzymes were toward the higher side in 40–60 years old patients (p<0.001).Serum Globulin levels were not significantly varied between gender and age groups. Calculated A:G ratio was significantly (p<0.001) lower in younger patients (≤40 years).

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On comparing the mean value of LDH between male and female patients, it is evident from the statistical results that the values were high in both genders, with more elevated LDH 636.7U/L (SD=671.2) in males and 543.9 U/L (SD=304.1) in females with a non-significant p=0.121. Serum LDH was high in all three age group subjects with the highest values 649.6 IU/L (SD=748.0) being recorded in, 40-60-year-old patients, followed by 635.2 IU/L (SD=400.8) in more than 60 years and 470.5 IU/L (SD=260.2) in less than 40 years old patients with a statistically significant p<0.001. The inflammatory marker D-Dimer was much above the normal reference range in both genders, but females showed remarkably high values 792.7ng/ml (SD=1863.8) compared to males 639.1ng/ml (SD=1615.2). Age wise comparison showed higher D-Dimer mean 889.8ng/ml (SD=1817.6) in the elderly patients above 60 years, followed by 665.0ng/ml (SD=1774.1) in ages 40-60-year range and 509.6ng/ml (SD=1377.9) in the patients below 40 years of age. Thus, D-Dimer was found to have a strong association with COVID-19 with a statistically significant p<0.001.

Liver enzymes were toward the higher side in 40–60 years old patients with a statistically significant p<0.001. Serum total protein levels were toward lower margin of the reference in our study patients. Albumin was below normal in all ages, the lowest of 3.3g/dl (SD=0.4) in patients between 40 and 60 years. Serum Globulin level was elevated most in females 4.3 gm/dl and 3.9 gm/. dl in older patients. Calculated A:G ratio was statistically significant with p<0.001 at 95% CI. In males and in smaller age group subjects (40 years and less), the A:G ratio was appropriately distributed as 1.17 and 1.4 respectively. In females and patients above 60 years the estimated AGR was 0.81 and 0.87, proved to be statistically significant (p <0.001)

Table 3: Exploratory linear mixed models for each biochemical parameter to estimate average change from day one

Biochemical parameters	Average change from day 1				
	On day 7 (95%CI)	P-value		On day 14 (95%CI)	P-value
LDH (U/L)	-52.1 (-137.4 to 33.4)	0.233		-255.4 (-407.2 to -103.6)	0.001
TB (mg/dl)	-0.3 (-0.4 to -0.2)	p<0.001		-0.4 (-0.5 to -0.3)	p<0.001
DB (mg/dl)	0.1 (0.08 to 0.2)	p<0.001		-0.02 (-0.08 to 0.05)	0.636
IB (mg/dl)	37.5 (33.9 to 41.1)	p<0.001		25.3 (18.1 to 32.6)	p<0.001
PT (U/L)	-6.7 (-12.8 to -0.6)	0.031		-14.8 (-23.1 to -6.5)	p<0.001
OT (U/L)	23.5 (17.8 to 29.2)	p<0.001		35.9 (26.6 to 45.3)	p<0.001
ALP (U/L)	-61.2 (-65.3 to -57.0)	p<0.001		-61.7 (-69.6 to -53.7)	p<0.001
TP (g/dl)	-3.8 (-4.8 to -2.8)	p<0.001		-4.1 (-5.3 to -2.7)	p<0.001
A (g/dl)	-0.7 (-0.8 to -0.5)	p<0.001		-0.6 (-0.8 to -0.4)	p<0.001
G (g/dl)	-1.7 (-1.8 to -1.6)	p<0.001		-1.8 (-2.0 to -1.6)	p<0.001
A:G	42.4 (38.2 to 46.6)	p<0.001		26.2 (17.5 to 34.9)	p<0.001
Ur (mg/dl)	-33.5 (-36.8 to -30.1)	p<0.001		-34.2 (-38.9 to -29.5)	p<0.001
Cr (mg/dl)	4.1 (2.5 to 5.7)	p<0.001		7.1 (3.8 to 10.4)	p<0.001
UA (mg/dl)	131.6 (128.0 to 135.2)	p<0.001		132.9 (125.5 to 140.2)	p<0.001
Na+(mmol/L)	-134.4 (-135.2 to -133.7)	p<0.001		-134.8 (-136.3 to -133.4)	p<0.001
K+(mEq/L)	101.3 (100.0 to 102.6)	p<0.001		99.4 (97.2 to 101.5)	p<0.001
Cl-(mmol/L)	1.2 (-0.4 to 2.8)	0.127		-0.7 (-3.2 to 1.9)	0.611

Models were adjusted for age and gender; CI: confidence interval; LDH: Lactate Dehydrogenase; DD: D-Dimer; TB: Total Bilirubin; DB: Direct Bilirubin; IB: Indirect Bilirubin; SGPT: Serum Glutamate Pyruvate Transaminase; SGOT: Serum Glutamate Oxaloacetate Transaminase; ALP: Alkaline Phosphatase; TP: Total Protein; A: Albumin; G: Globulins; A:G: Albumin:Globulin; Ur: Urea; Cr: Creatinine; UA: Uric Acid; Na+: Sodium; K+: Potassium; Cl-: Chloride

Table 3 showed the average change on day seven, and fourteen from day one using linear mixed models adjusted for age and gender. Lactate Dehydrogenase showed 255.4 unit average decrease on day fourteen (p=0.001) compared to day one however, day seven decreases was not statistically significant (p=0.233). Most biochemical variables have significantly changed at both time points compared to day one estimates except for Chloride (day seven p=0.127; day fourteen p=0.611), and Direct Bilirubin (day fourteen p=0.636).

Discussion

The notorious coronavirus since its outbreak in December 2019 has duped the human race and the infectious nature of the virus has aided its spread to millions of people around the world. In this scenario, clinicians and scientists are exploring various diagnostic test strategies to conquer the small smart virus, 'The coronavirus'.

The potential to create a dependable prognosis at the time of COVID-19 patient's admission will avert needless hospitalization, and help secure optimum use of resources. In COVID-19 patients, it is crucial to diagnose early and determine a good prognosis. This study was planned to render help to clinicians in predicting the severity, the need for hospitalization and to detect risk of multiple organ functioning.

We evaluated the data of 483 successively hospitalized COVID-19 patients on day 1, 7 and 14. In this study 66.3% of patients were males similar to a study by Maria Martinez et al., which reported 66.5% male patients [12]. To justify, George M et al., has stated a preexisting biological difference in the immune systems between men and women, which might affect the ability to fight infection including SARS-2-CoV-2 [13]. Ghazeeri G. et al., Elgendy IY. et al., portrayed the role of estrogen receptors in females who act as mediators and block the viral infection, thus rendering them more resistant to infections than men [14, 15]. Zhao Y et al., postulated higher

expression of ACE-2 receptors for coronavirus in men [16] with life style changes, such as high rate of drinking and smoking among men compared to women [16, 17].

Coagulation Function Tests

Our study outcome showed certain abnormal biochemical indicators with a significant difference in 1st, 7th, and 14th days, respectively. To evaluate the extent of coagulation function in COVID-19, we estimated the levels of serum LDH and D-Dimer. High levels of LDH and D-Dimer were reported in Co-V-2 infection on the day of hospitalization. Serum LDH was elevated by 2.5 times than upper reference range in odds of disease severity with a highest value 606.3U/L on the first day, which gradually declined to 506.4U/L on 7th day and further to 466.9U/L on 14th day (Table1). Thus, our Patient's data analysis demonstrates deep association between serum LDH levels and coronavirus disease severity. Brandon M Henry et al., reported >6-times increase in LDH levels in severe disease and >16-fold raise in case of patients mortality [18].

LDH is an intracellular enzyme present in all cells of most organs, catalyzes the interconversion of pyruvate and lactate, with concomitant interconversion of NADH and NAD^+. The enzyme exists in humans in five isoenzyme forms; LDH-1 to LDH-5 (LDH-1 in cardiomyocytes, LDH-2 in reticuloendothelial system, LDH-3 in pneumocytes, LDH-4 in pancreas and kidneys and LDH-5 in liver and striated muscle). Decades of work confirm the association of raised LDH levels with multiple organ injury and up-regulation of glycolysis leading to decreased oxygenation, increased lactate concentration thus creating an acidic pH in infection and tissue injury [19]. Since, LDH-3 isozyme is present in lungs, in COVID-19 patients, there is increased release of LDH into blood circulation, as a form of pneumonia, often resulting in acute respiratory distress syndrome (ARDS), the hallmark of COVID-19.

Concerning demographic factors, serum LDH levels were most elevated in males (636.7U/L) and in patients between 40 and 60 years of age (649.6U/L), with a statistical significant p<0.001(Table2). LDH of comparison on all three mentioned days of the study, showed an increasing negative change (-255.4 at CI of 95%), a decline on 14th day with a significant p=0.001 (Table 3) compared to the 7th day (-52.1 at CI of 95%). Thus, LDH levels help understand better patient recovery after hospitalization and proper treatment regimen by 14th day.

Thus, our study results demonstrate a robust association between higher LDH levels measured at the time of admission and subsequently reduction to in the levels of LDH during hospitalization, suggestive of better recovery; more so, as our study subjects were of mild to moderate illness. The strong correlation between COVID-19 and LDH (Table 2) is narrated by the fact that LDH is a biomarker of lung damage [20] and lower respiratory tract is primarily infected by COVID-19.

During the COVID-19 pandemic, D-Dimer is estimated to exclude pulmonary embolism or deep vein thrombosis. On analyzing the data, significantly higher levels of D-Dimer are documented, possibly due to the activation of coagulation function. Present study analysis reveals strong association of serum D-dimer values with COVID-19 severity. Though most subjects were of mild to moderate illness, high D-Dimer levels (693.7ng/ml) at the time of admission (Table1) (Reference range <500ng/ml) were noted. A recent study by Hai-Han Yu et al., also proposed elevated levels of D-Dimer in COVID-1progression, due to abnormal coagulation function [21]. In a cohort study of 2377 consecutive coronavirus-infected patients, 76% patients had raised D-Dimer levels at the hospital presentation in a severe form of infection [22].

D-Dimer was found to be elevated in our study group, with higher levels noted in female patients (792.7ng/ml) and in above 60 year's patients (889.8ng/ml) (Table2). Thus, increased D-Dimer in the elderly COVID-19 patients warns rapid deterioration of health; likely, reflecting coagulation activation from infection/ sepsis, cytokine storm and patient heading towards organ failure. Findings of Snijders D et al., express elevated levels of D-Dimer indicative of higher risk of coagulopathy, mortality and lower levels may suggest good prognosis [23]

When cross linked fibrin gets degraded, D-Dimer formation takes place, which acts as a global marker of coagulation activation and fibrinolysis and heightened risk of thrombolysis [24]. In COVID-19, various micro and macro clots are present in multiple vascular beds, wherein the D-Dimer then induces acute pulmonary pneumonia and pathogenic effects [25, 26].

In the data study D-Dimer values were available only from first day of patients hospitalization so could not associate further results with disease severity. A clear cut association of D-Dimer with COVID-19 illness is noticed even from the initial day of the test results. Thus, striking evidence greatly supports the prognostic ability of D-Dimer and its evaluation for timely medical intervention to reduce thrombotic risk and adverse outcomes. Our results boost the scientific rationale for clinical trials to reduce adverse outcomes.

Liver Function Tests

To assess the liver functions in COVID-19 patients, we assayed a series of biochemical tests - total proteins with its two components-albumin and globulin [27] in addition to the enzymes. Mean values of serum total protein and albumin was towards lower normal range on the initial day of hospitalization with a subsequent decrease in total protein levels to 5.5 gm/dl and 5.3 gm/dl; and albumin to 2.9 gm/dl and 2.7gm/dl, suggestive of hypoalbuminemia on 7th and 14th day respectively. (Table1). Our results were similar to Gavriela M. Feketea et al.,study with decline in Albumin in COVID-19 patients, reflecting the underlying kidney and liver problems [28].

Globulins are fractions of plasma proteins named as alpha, beta, gamma globulins. The gamma globulins exhibit immunological role acting in defense mechanism of the body and rise in microbial infections [28], including corona virus infection. Our result shows elevated values of globulins 4.3gm/dl in females and 3.9gm/dl in patients above 60 years of age. Higher levels could be due to age related co morbid conditions in the older patients. On calculating albumin to globulin ratio (AGR) according to the following formula: AGR=albumin/globulin, results were lower AGR in females and in elderly patients as 0.814 and 0.872 respectively (Table 2); inversely proportional to globulins. Lower AGR could be justified with the fact that albumin was low and globulin high. A study demonstrated AGR lower than 1 in COVID-19 patients with other co morbidities mainly due to hypoalbuminemia or albumin as such declines with age [28]. AGR might be a good indicator, reflects, a combination of nutritional as well as the inflammatory status. Another study by Qin J et al., states to consider AGR as an index to predict the prognosis of inflammatory diseases thus suggesting low AGR in susceptible infectious state [29].

Liver enzymes play a crucial role in disease processes, they provide immense help in achieving a conclusive diagnosis and serial estimations to assess disease prognosis [27]. Though the liver enzymes show derangement in most studies, our results found both AST and ALT within the normal limits in all hospitalized patients with a statistically significant p<0.001 (Table2). A study by Huang C et al., [30] showed a significant association between high levels of transaminase in COVID-19 patients. Probable reason for discrepancy could be the mild to moderate type of illness of our study patients. Thus, in non severe COVID-19 infection, liver biomarkers do not show much alteration.

Renal Function Tests

In the current study kidney markers, creatinine and urea were found to be in reference limits in our patients thus suggestive of normal kidney functions in mild to moderate COVID-19 infectious condition, with statistically significant p value <0.001 (at 95% CI) . Few researches reveal renal abnormalities in critical viremic infection along with other organ injury. An Italian report about 25-30% of COVID-19 patients are prone to develop acute kidney injury (AKI); marked by elevated serum creatinine levels thus causing direct infection to the renal tubular cells, which expresses the ACE2 receptors on their cellular surface [31]. Enrolled patients revealed a better prognosis and

recovery. Thus, renal parameters were within the normal range in our hospitalised COVID-19 patients. Our data analysis of the serum electrolyte demonstrated normal levels with slight alteration in sodium 135.4 mmol/L and potassium 5.1mEq/L in 40-60 years of age patients. Thus, sodium and potassium showed an inverse relationship with significant p<0.001(Table 2). Lippi G et al., stated hyponatremia and hypokalemia in patients with severe COVID-19 [32]. Our findings of the study did not report any such condition, probably because of the less severe disease condition.

Limitations

There are several limitations of our study. First, because of the novelty of the coronavirus outbreak, the number and diversity of biochemical studies on COVID-19 patients is small and the majority from Mainland China. Second, the available data were from mild to moderate COVID-19 patients; during declining phase of the first wave of the pandemic in Indian province; between September 2020-November 2020 compared to the initial infectious spread (March to July 2020), and patients with critical COVID-19 illness were not assessed. Since the patients were of mild to moderate illness, we could not assay D-Dimer on 7th and 14th day.

Third, due to the sudden outbreak of the disease and less available literature, we excluded the pre existing, co morbidities in COVID-19 patients. Fourth, due to the contagious nature of the disease, we failed to take the history of patients from the case sheets of the hospital to differentiate between the symptomatic and asymptomatic cases, which can be initiated for future research.

Further investigation must verify the findings from a prognostic perspective to determine parameters that can aid the differentiation between non-severe and severe COVID-19 cases. By the inclusion of studies with raw mean data, we can avoid discrepancies that could occur because of varying cut-offs of laboratory parameters across studies. Further research is warranted to explore the association of other biochemical markers with COVID-19 severity.

Conclusion

This cohort study conducted on confirmed COVID-19 patients reveals a strong direct correlation between serum LDH levels and the disease. On comparing the mean values of LDH on first to fourteenth through the seventh day of hospitalization, a better prognosis is evident in the participating patients.

Nevertheless, our cohort study demonstrated high levels of D-Dimer, even in mild to moderate COVID-19 patients. Thus, evident that elevated D-Dimer levels might have a higher risk of severe infection, providing a timely reminder to physicians to pay early clinical attention to those COVID-19 patients. There by, serum LDH and D-Dimer are directly associated with all forms of COVID-19.

Since the available data of study patients were of mild to moderate type of infection, when the first wave of the pandemic was downside, there was no demarcated alteration in other biochemical; except for a slight decrease in serum albumin and total protein levels.

Our retrospective study is novel in a way since we analyzed the biochemical data of confirmed COVID-19 patients on three different days of hospitalization; 1st, 7th, and 14th day, which till date has not been studied.

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