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ORIGINAL ARTICLE

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Biochemical and Inflammatory Markers in Covid-19 Patients of a Tertiary Care Hospital at North Karnataka – A Descriptive Study

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Background: Covid-19 has emerged as a public health crisis. Biochemical and laboratory parameters play a role in understanding the disease process.

Aims and objectives: 1. Understanding the demographic pattern of Covid-19 disease in the study population. 2. To analyze biochemical and inflammatory markers in Covid-19 patients. Study includes the data collected from Covid -19 Hospital laboratory of a tertiary care hospital at KIMS (Karnataka Institute of medical sciences, Hubballi, Karnataka, India). Data was collected from March to October 2020 (Total 7800 patients had Covid-19 during this period). The present study includes the data from the 4478 Covid-19 patients of age group ranging from 1year to 99 years. Newborn to Covid-19 positive mothers and patients whose complete data was not available were excluded from the study. The parameters included in the study are CRP(c reactive protein), Ferritin, LDH (Lactate dehydrogenase), Troponin-I, and D-dimer.

Results: The levels of CRP, LDH, Ferritin were significantly increased in males compared to females. There was a significant difference in the levels of CRP, LDH, Ferritin, and D-dimer among different age groups, but no such statistical difference was found for Troponin-I.

Conclusion: This study suggests the possibility of increase in severity of the disease with increasing age. This possibility of association between age and severity of disease was earlier studied only on small sample size. This study also suggests that disease severity is more common in males than in females.

Key words: CRP, LDH, Troponin-I, Ferritin, D-dimer

The Covid-19 pandemic is a major challenge faced by the health care sector worldwide. Covid-19 is caused by severe acute respiratory syndrome Corona Virus 2 (SARS –CoV-2)[1]. The covid-19 disease has rapidly spread worldwide and the World Health Organization (WHO) has declared this disease as pandemic on 11th March 2020[2]. WHO has reported 129 215 179 confirmed cases of Covid-19 and 2 820 098 deaths worldwide till 02/04/2021. In India 12 303 131confirmed cases and 163 396 deaths are reported till 02/04/2021[3]. Currently, real-time reverse transcription PCR is the most reliable test to diagnose Covid-19 around the world[1]. In the management of Covid-19 disease many laboratory markers are used like lymphocyte count, neutrophil count, neutrophil-lymphocyte ratio, LDH, Troponin-I, Ferritin, D-dimer, CRP, ESR (Erytrocyte sedimentation rate), procalcitonin, AST (Aspartate Transaminase), Interleukin-6. In our study, we planned to study CRP, LDH, Troponin-I, Ferritin, D-dimer. C reactive protein is an acute phase reactant and a plasma protein synthesized in the liver. CRP levels are used as a biomarker for many inflammatory conditions[4]. LDH is present in most of the cells of the body. It has many isoenzyme forms and is mainly present in the liver, heart, lungs, muscles, kidneys, and blood cells. LDH is found to be increased in acute and severe lung damage[5]. Increased levels of LDH are also found in interstitial lung infections[6]. Troponin-I is a protein that acts by binding to actin and inhibiting the interaction between actin and myosin[7]. The increase in the levels of Troponin-I is directly correlating with the degree of myocardial inflammation[8]. Ferritin is a protein involved in iron storage and is involved in iron homeostasis[9]. Serum ferritin is also considered an acute phase reactant and is found to be increased in chronic inflammation[10]. D-dimers are by-products said to be derived from the lysis of cross-linked insoluble molecules of fibrin[11]. Earlier D-dimer assays were restricted to the diagnosis of Disseminated Intravascular coagulation, but in recent years D-dimer is used as a diagnostic and prognostic indicator of thrombotic status[12]. Hence we planned to study these parameters in patients with Covid-19 disease.

The study results will provide an important baseline for planning a proforma for the diagnostic management of Covid-19 patients by health care authorities. This will facilitate the allocation of resources in the proper direction.

So far the studies published on the laboratory markers are mostly Meta-analysis or review articles, the studies included in the meta-analysis and reviews were from different geographical backgrounds and also these studies were done on a small group of the population, or the sample size was very less. As our study includes large sample size and also the population under study mostly comes from a similar geographical area, the results obtained from the study can be extended to the population at large and will help in improving the existing knowledge about the laboratory markers in Covid-19 disease.

MATERIALS AND METHODS

This study included the data of patients from a tertiary care Covid-19 center of KIMS Hubballi. The majority of patients admitted belonged to the Dharwad district of Karnataka state (Dharwad district population - Approximately 19.5 lakh) and some patients were referred from surrounding districts of North Karnataka. This study is a descriptive retrospective study. Data is collected from the Covid-19 hospital laboratory at KIMS Hubballi from May 2020 to October 2020. (Total 7800 patients had Covid-19 during this period). The present study includes the data from the 4478 Covid-19 patients of age group ranging from 1year to 99 years. Exclusion criteria: newborn to Covid-19 positive mothers, patients whose complete data was not available.

The parameters included in the study are CRP, Ferritin, LDH, Troponin-I, and D- dimer. CRP and LDH were estimated by micro-slide method, Ferritin and Troponin-I were estimated by Chemiluminescence immunoassay and D-dimer was estimated by turbidimetric immunoassay. Ethical committee clearance was obtained from the institutional ethical committee on 23-12-2020.

Statistical analysis: This is a retrospective demographic study. p-value < 0.05 is considered to be significant and $p < 0.001$ is considered as highly

significant. Statistical analysis was done using SPSS software and Medcalc software.

RESULTS

Table I shows demographic details of CRP, Table 2 shows demographic details of LDH, Table 3 shows demographic details of Troponin-I, Table 4 shows demographic details of Ferritin, Table 5 shows demographic details of D-dimer, Table 6 shows demographic characteristics of different parameters in Females and Males. Table VII shows comparison tables of different parameters (ANOVA table). We found that statistical significance was found among the different age groups for all parameters except Troponin-I (Table 7). All parameters were found to be significantly increased in males compared to females except for Troponin-I and D-dimer (Table 6).

Table 1: Demographic details of CRP

Sl no	AGE (yrs)	Mean (mg/L)	S.D	S.E	95 % confidence (Lower Bound)	95 % confidence (Upper Bound)	Min	Max
1	(1-15) n = 54	289.66	301.05	40.96	207.493	371.840	.0	1001.0
2	(16-25) n = 226	226.41	206.25	13.71	199.376	253.447	.0	1001.0
3	(26-35) n = 453	252.01	191.12	8.97	234.370	269.665	.0	1001.0
4	(36- 45) n = 627	300.53	231.29	9.23	282.393	318.672	.0	1001.0
5	(46 – 55) n = 1011	357.88	252.37	7.93	342.308	373.459	.0	1001.0
6	(55 – 65) n = 1220	365.12	251.37	7.20	350.986	379.255	.0	1001.0
7	(> 65) n = 887	387.67	294.68	9.89	368.254	407.092	.0	3198.0
8	Total n = 4478	339.55	256.03	3.82	332.057	347.059	.0	3198.0

Normal range of CRP: 1.0 to 3.0mg/L

Table 2. Demographic details of LDH

Sl no	AGE (yrs)	Mean (U/L)	S.D	S.E	95 % confidence (Lower Bound)	95 % confidence (Upper Bound)	Min	Max
1	(1-15) n = 54	289.66	301.05	40.96	207.493	371.840	.0	1001.0
2	(16-25) n = 226	226.41	206.25	13.71	199.376	253.447	.0	1001.0
3	(26-35) n = 453	252.01	191.12	8.97	234.370	269.665	.0	1001.0
4	(36- 45) n = 627	300.53	231.29	9.23	282.393	318.672	.0	1001.0
5	(46 – 55) n = 1011	357.88	252.37	7.93	342.308	373.459	.0	1001.0
6	(55 – 65) n = 1220	365.12	251.37	7.20	350.986	379.255	.0	1001.0
7	(> 65) n = 887	387.67	294.68	9.89	368.254	407.092	.0	3198.0
8	Total n = 4478	339.55	256.03	3.82	332.057	347.059	.0	3198.0

Normal range: 140UL to 280U/L

Table 3. Demographic details of Troponin-I

Sl no	AGE (yrs)	Mean (ng/ml)	S.D	S.E	95 % confidence (Lower Bound)	95 % confidence (Upper Bound)	Min	Max
1	(1-15) n = 54	0.334	2.380	0.323	-0.315374	0.983892	0.000	17.50
2	(16-25) n = 226	0.484	0.272	0.018	0.012641	0.084208	0.000	3.710
3	(26-35) n = 453	0.299	0.175	0.082	0.013774	0.046146	0.000	2.780
4	(36- 45) n = 627	0.179	3.013	0.120	-0.056718	0.416252	0.000	74.80
5	(46 – 55) n = 1011	0.257	2.641	0.831	0.093906	0.420108	0.000	47.00
6	(55 – 65) n = 1220	0.654	14.07	0.403	-0.136558	1.446452	0.000	485.0
7	(> 65) n = 887	1.950	31.70	1.064	-0.138890	4.044038	0.000	772.0
8	Total n = 4478	0.657	16.01	0.239	0.188336	1.127138	0.000	772.0

Normal range < 0.04ng/ml

Table 4. Demographic details of Ferritin

Sl no	AGE (yrs)	Mean (micro g / L)	S.D	S.E	95 % confidence (Lower Bound)	95 % confidence (Upper Bound)	Min	Max
1	(1-15) n = 54	167.2	256.6	34.92	97.253	237.340	.0	1001
2	(16-25) n = 226	149.3	246.1	16.37	117.051	181.569	.0	1406
3	(26-35) n = 453	200.0	385.5	18.11	164.406	235.598	.0	6387
4	(36- 45) n = 627	258.9	299.3	11.95	235.495	282.445	.0	1001
5	(46 – 55) n = 1011	308.9	313.7	09.86	289.578	328.309	.0	1001
6	(55 – 65) n = 1220	340.3	355.3	10.17	320.364	360.281	.0	5305
7	(> 65) n = 887	375.4	341.1	11.45	352.929	397.897	.0	1001
8	Total n = 4478	339.3	339.3	05.07	292.934	312.819	.0	6387

Normal range: Males: 24-336micro g/ L, Females: 11- 307 micro g/L.

Table 5. Demographic details of D-dimer

Sl no	AGE (yrs)	Mean (ng/ml)	S.D	S.E	95 % confidence (Lower Bound)	95 % confidence (Upper Bound)	Min	Max
1	(1-15) n = 54	214.6	823.2	112.02	-10.006	439.376	.0	5574
2	(16-25) n = 226	583.7	1679.5	111.71	363.602	803.903	.0	13300
3	(26-35) n = 453	486.7	1410.7	66.282	356.511	617.030	.0	17900
4	(36- 45) n = 627	927.0	2756.2	110.07	710.906	1143.222	.0	26100
5	(46 – 55) n = 1011	1259.8	4744.4	149.21	967.087	1552.699	.0	90500
6	(55 – 65) n = 1220	1278.2	5149.2	147.42	988.974	1567.431	.0	118000
7	(> 65) n = 887	1614.9	6620.9	222.310	1178.678	2051.311	.0	97200
8	Total n = 4478	1163.6	4744.8	70.90	1024.668	1302.690	.0	118000

Normal range: D-dimer <250ng/ml

Table 6. Demographic characteristics of different parameters in Females (F) and Males (M)

	Gender	CRP	LDH	Troponin-I	Ferritin	D-dimer
Mean	F	32.127	313.032	1.0285	192.002	979.871
	M	38.138	352.222	.480650	355.808	1251.429
		p < 0.001	p < 0.001	p = 0.284	p < 0.001	p =0.073
N	F	1447	1447	1447	1447	1447
	M	3031	3031	3028	3031	3031
S D	F	36.7004	251.5406	19.2657	251.1190	4230.2103
	M	39.8437	257.2268	14.2027	362.4976	4970.1109
S E of Mean	F	0.9648	6.6126	0.5066425	6.6015	111.2059
	M	0.7237	4.6722	0.2581037	6.5843	90.2762

Table 7. Comparison tables of different parameters (ANOVA table)

		Sum of squares	Df	Mean square	F	significance
CRP	Between Groups	323407.532	6	53901.255	37.249	0.000
	Within Groups	6469800.494	4471	1447.059		
	Total	6793208.025	4477			
LDH	Between Groups	10644244.92	6	1774040.820	28.044	0.000
	Within Groups	282834113.4	4471	63259.699		
	Total	293478358.3	4477			
TROP I	Between Groups	2056.248	6	342.708	1.337	0.237
	Within Groups	1145176.366	4467	256.362		
	Total	114732.615	4477			
FERRTIN	Between Groups	18740072.98	6	3123345.397	28.104	0.000
	Within Groups	496881482.9	4471	111134.306		
	Total	515621555.9	4477			
D-DIMER	Between Groups	573338835.7	6	95556472.62	4.263	0.000
	Within Groups	1.002E+11	4471	22415938.71		
	Total	1.008E+11	4477			

DISCUSSION

The entire world is hit by the Covid-19 pandemic. Several laboratory parameters were found to alter in Covid-19 disease. We studied the following lab parameters CRP, LDH, Ferritin, D-dimer, and Troponin-I. These parameters play an important role in the management of Covid-19. In this study, we have compared the mean values of the different laboratory parameters in males and females (Table 7). We found

that the given parameters were found to be increased when compared to the normal reference values both in males and females (Table I to V).

An increase in CRP levels may reflect the severity of lung disease[13].In our study, we found that the CRP levels were more than the normal reference range in patients with all age groups (Table I). We also found that CRP levels increased as the age increased except for patients with age group (1-15 years), which might be due to the small sample size of patients in this age group and it may not reflect the actual mean. The lowest level of CRP was found in the 16-25 age group (mean CRP = 17.92) and the highest level was found in the age group above 65 years (mean CRP = 44.13). The increase in CRP levels with an increase in age might indicate that the severity of the Covid-19 disease may be seen more commonly in the older age group, as it has been shown in many studies that an increase in CRP levels predict the severity of the disease[15]. In a study conducted by Trupti et al[14] authors showed a comparison of patients with normal CRP and abnormal CRP levels in different age groups and the results were similar to our study. We also found that CRP levels in males were significantly increased when compared to females ($p<0.001$). A study conducted by Trupti Bajpai[14] et al on 556 patients found that there was no significant difference in CRP values in males and females.

LDH is said to be a good marker of vessel permeability in immune-mediated lung injury[16]. In our study, we found that the LDH levels were more than the normal reference range in patients of all age groups (Table II). We also found that LDH levels increased as the age increased except for patients with age groups (1-15 years). The lowest level of LDH was found in the 16-25 age group (mean LDH = 226.41) and the highest level was found in the age group above 65 years (mean LDH = 387.67). A Meta-analysis conducted by Lukasz Szarpak et al concluded that LDH level can be used as a COVID-19 severity marker and is a predictor of survival[17]. We also found that LDH levels in males were significantly increased when compared to females ($p<0.001$). This observation was also seen in a study conducted by Jin Hu[18] et al which showed LDH levels were increased in males compared to females ($p<0.01$).

An increase in Troponin-I levels is commonly seen in Covid-19 patients and is associated with fatal outcomes[19]. In our study, we found that the Troponin-I levels were more than the normal reference range in patients of all age groups (Table III). We also found that the lowest level of Troponin-I was seen among the age group 36-45 years (mean Troponin = I0.179) and the highest value was found in the age group >65 years (mean Troponin = 1.950). Hence we may conclude that patients above 65 years are more prone to developing cardiac complications in Covid-19. It was also observed that even though there was a difference among the age groups, it was not statistically significant (Table VII). A meta-analysis conducted by Bing – Cheng[20] found that elevated Troponin on admission was associated with a higher risk of subsequent death (risk ratio 2.68, 95%CI 2.08 – 3.46) after adjusting confounders in multivariable analysis. In this study, we found that Troponin-I levels were elevated in males compared to females but it did not show any statistical significance ($p = 0.284$), this finding was similar to the study conducted by Simen Ozylmaz[21], where there was no statistical significance found ($p = 0.913$).

Elevated levels of Ferritin are found to be associated with disease severity in patients with Covid-19[22]. In our study, we found that the ferritin levels were increased more than the reference range in patients with age groups above 46 years (Table IV). Among these age groups, the highest increase was seen in patients above 65 years (mean ferritin = 375.4 microgram/L). A study conducted by Zhi Lin[23] et al concluded that a high level of serum ferritin is an independent risk factor for the severity of Covid-19 and assessing serum ferritin levels during hospitalization may be important to recognize high-risk individuals with Covid-19. Hence we may infer that the patients with age group above 65 years may be at risk for developing severe Covid-19. In this study, we found that serum ferritin levels were more elevated in males compared to females and the difference was found to be statistically significant ($p<0.001$). A study conducted by O. Gandini[24] et al showed a significant difference in serum ferritin levels in males and females.

Estimating the circulating levels of D-dimer is a sensitive indicator to detect thrombotic states in Covid-

19[25].In our study, we found that D-dimer levels were increased more than the normal reference range in patients with all age groups except for patients less than 15 years of age. The highest level of D-dimer was observed in the age group above 65 years (Table V) (mean D dimer = 1614.9ng/ml). A systemic review and meta-analysis conducted by Panagiotis et al[26] found that D-dimer concentrations in patients with severe Covid-19 are significantly higher compared to those with nonsevere forms. Thus we may conclude that the patients with age group above 65 years are more prone to developing a severe form of Covid-19. We also found that D-dimer levels were higher in males compared to females, but there was no statistically significant difference found (p=0.073). A study conducted by Kumar Sharp[15] showed that females are at a higher risk of developing thrombotic disorders than men.

CONCLUSION

This study suggests the possibility of increase in severity of the disease with increasing age. This possibility of association between age and severity of disease was earlier studied only on small sample size. This study also suggests that disease severity is more common in males than in females.

LIMITATIONS

The data was collected from the laboratory only. Thus the study lacks clinical correlation of the severity. A study of these parameters along with clinical correlation may give more insight or information into predicting severity, complications, and mortality among Covid-19 patients.

CONFLICTS OF INTEREST

All authors have declared that they do not have any conflict of interest for publishing this research.

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