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Predictive Role of Haematological and Biochemical Markers in Assessing Covid -19 severity in Southern Ethnics

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Abstract

Background: There is a high mortality and morbidity due to COVID-19 infection worldwide. Hence, there is a need for potential biomarker analysis and its role in early management of covid-19. Therefore, this study aimed to analyse and find out correlation of various biochemical and haematological markers to assess the severity.

Methodology: This cross-sectional study was conducted at Tirunelveli medical College, for a period of one year, 154 covid-19 confirmed patients were included. The disease severity was correlated with various biochemical and haematological markers.

Results and statistics: Statistical analysis was done by SPSS 22. Among the study population 88 patients were males (57.8%) and (42.2%) 66 patient were females. The age group between 50 -69 years were affected more. Increasing age had positive correlation with Neutrophil and Lymphocyte count, Urea, Glucose, Ferritin and ALP. Sex had positive correlation with Ferritin (P< 0.05). Mean and SD of WBC 7440.06 + 3298.731, Mean and SD of Glucose 134+ 71.7, Mean and SD of LDH 313.26+ 213.45, Mean and SD of D-dimer 427.1+ 526.49, Mean and SD of Ferritin 300+303.07. Total count had positive correlation with LDH, Ferritin and D-dimer (P<0.05). Neutrophil count had significant correlation with ESR, CRP and LDH. Lymphocyte count significantly correlated with LDH; Glucose had significant correlation with age, urea and creatinine; Urea had significant correlation with age, LDH and ferritin (P<0.05).

Conclusion: This study concludes that elevated Neutrophil count, Ferritin, CRP, LDH, Glucose, Renal parameters and D dimer were associated with disease severity and prognosis in Covid patients.

Keywords: Covid-19, Haematological Markers, Biochemical Markers, C Reactive protein, Ferritin, CBC

Introduction

COVID-19 is an infectious disease caused by the SARS-CoV-2 virus, which had given rise to a global sanitary emergency. Patients with coronavirus disease demonstrated a series of clinical symptoms, including raised body temperature, cough, headache, nausea, vomiting, anorexia, diarrhea, dyspnea, multiple organ dysfunction. An enormous proportion of infected patients reported with mild symptoms of the disease, some patients progressively developed severe complications such as sepsis, acute respiratory failure, metabolic acidosis, heart failure, acute and chronic kidney injury, hypoxic encephalopathy, which leads to death [1]. Considering its high transmission and infectivity rate, WHO declared it as an emergency of public health concern on March 31, 2020[3]. During the initial phase of the disease outbreak, the mortality rate was higher among the elderly [1]. Hence, the early identification of severe illness and risk factors would support the clinicians

to initiate appropriate remedial measures and helps to control mortality and morbidity rate [4]. Earlier existing literature reported changes in the covid 19 patients' biochemical parameters, including lymphocyte count, neutrophil count, and D-dimer status.[2,5] Another study reported changes in inflammatory markers in patients with COVID-19, including C-reactive Protein (CRP), Erythrocyte Sedimentation rate (ESR), and Interleukin-6.[1]. Similarly, some studies had reported lymphocytopenia, high serum Glucose, Gamma-Glutamyl Transferase (GGT), high Lactate Dehydrogenase (LDH) in more COVID-19 patients.[6]. Furthermore, some other studies also demonstrated an increased level of serum urea, cardiac troponin, creatinine kinase, D-dimer, CRP, LDH, IL-6, and lower level of lactic acid levels and lymphocytes[7,8]. So, there is a need for early diagnose and initiation of early treatment of covid 19 infection to prevent early morbidity and mortality. In our study, we aimed to analyse the Heamotological and Biochemical markers in Covid- 19 infected patients in our region.

Materials and Methods

Study design and participants:

In this cross sectional study, 154 RTPCR confirmed COVID-19 patients admitted from August 2020 to July 2021, at Government Tirunelveli Medical College and Hospital, Tirunelveli, Tamil Nadu were included. Diagnosis of study COVID-19 and clinical classification according to the new coronavirus pneumonia diagnosis and treatment plan (trial version 7) developed by WHO and ICMR India was used in the study[5]. The study was approved by the Institutional Ethics Committees at Tirunelveli Medical College and Hospital.

Sample collection: With all covid precautions and under aseptic precaution, 5 ml blood collected from the Covid-19 patient immediately after admission in Covid ward at Tirunelveli Medical College hospital. 2 ml of blood transferred to EDTA vacuitaner for haematological analysis, 3 ml of blood transferred to another vacutainer for Biochemical analysis. Immediately sample transferred to haematological and biochemical laboratory and analysis done. The Serum glucose estimated by GOD-POD method, Serum urea by Urease method, creatinine by Jaffey's method, AST and ALT by IFSC kinetic, Total Protein by Biuret method, Albumin by BCG method, ALP, C- reactive protein done with ERBO -360 ERBA and 640 fully automated analyser. D-dimer and haematological investigations including Complete Blood Count SYSMEX –XP 100 Prothrombin Time, activated plasma thromboplastin time were performed with ECL ERBA -412 and repeated according to clinical condition.

Statistical Analysis

Datas were transformed into a Microsoft Excel spreadsheet and analysed using Statistical Package for Social Sciences (SPSS) Version 22. Mean and Standard deviations were used for the comparison of continuous variables ensuring normal distribution. Pearson correlation was used to correlate various biochemical and haematological parameters. Categorical variables were given as frequency rates and percentages; continuous variables were defined using Mean, Median, and Interquartile range (IQR) values. The Chi-square test used for the categorical variables. In correlation analysis, Pearson correlation coefficient was used for the variables of normal distribution and Spearman correlation coefficient for those of skewed distribution. Receiver–operating characteristic (ROC) curve analysis was used to determine the optimum cut-off points of parameters for severe patients. A 2-tailed $P < 0.05$ was considered as statistically significant.

Results

In this study 154 hospitalized patients with confirmed COVID-19 were included. In our study the age of the covid patients ranged from 18 to 85 years were included. As per table 1, the age group between 50 -69 years were affected more (39 %) followed by 30 -49 years (34%),18 – 29 years (14%) and more than 70 years (13%). The Median age was 58 years. 88 patients were males (57.8%) and 66 patients (42.2%) were females. Age and sex of the study population had been correlated with various biochemical and haematological parameters. Figure 1, shows the serum level of glucose in various age groups and higher level was found in the age group between 40 -60 years. Females had more glucose level in comparison with male patients. According to Figure 2, CRP levels were more in males than females and raised in age group between 50 -60 years. Glucose and CRP had significant changes in covid positive patients than ESR and urea level

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(Table 2). Mean and SD of Age: 49.48+ 17.191, Mean and SD of WBC 7440.06 ± 3298.731, Mean and SD of Glucose 134+ 71.7, Mean and SD of LDH 313.26+ 213.45, Mean and SD of D-dimer 427.1± 526.49, Mean and SD of Ferritin 300±303.07, Mean and SD of Urea 37.9±26.41, Mean and SD of Creatinine 1.14±0.8, Mean and SD of CRP 24.9±41.3, Mean and SD of Sodium 140.59±595.9, Mean and SD of Potassium 5.2±6.2 (Table 3). Age had positive correlation with neutrophil count, lymphocyte count and ferritin level, Sex had no significant correlation with neutrophil count, lymphocyte count, CRP, D-dimer level Neutrophil count had positive correlation with Age, lymphocyte count, and CRP level. Lymphocyte count had positive correlation with Age, Neutrophil count, CRP, D- dimer level. C reactive protein level had a significant correlation with neutrophil and lymphocyte count. D – dimer level showed a significant correlation with lymphocyte count ($P < 0.05$). Ferritin level positively correlated with age (table 4). As per table 5, spearman's correlation, Total count had positive correlation with ferritin, glucose and creatinine, Neutrophil count had positive correlation with total count, lymphocyte count and creatinine, Lymphocyte count had positive correlation with total leucocyte count, neutrophil count and creatinine, D-dimer had positive correlation with aPTT and Creatinine. Ferritin had positive correlation with total leucocyte count and Creatinine. aPTT positively correlated with D – dimer($P < 0.05$). Glucose level showed significant correlation with total leucocyte count, neutrophil count, urea and creatinine level. Serum urea level showed significant correlation with glucose and creatinine level. Creatinine level showed positive correlation with Total count, neutrophil count, lymphocyte count, D-dimer level and ferritin level ($P < 0.05$). As per table 6, ESR had positive correlation with CRP. CRP had positive correlation with ESR and Direct Bilirubin level. Total bilirubin level had positive correlation with indirect bilirubin level. Direct bilirubin level had positive correlation with total bilirubin level, Indirect bilirubin and globulin ($P<0.05$). Indirect bilirubin had positive correlation with ALP, Globulin and total Bilirubin ($P<0.05$). Total protein, Globulin and bilirubin had significant changes ($P<0.05$).

Table 1: Distribution of age in COVID -19 patients

S. No	Age Group(years)	Numbers	Percentage
1	18- 29	22	14
2	30-49	52	34
3	50-69	60	39
4	> 70	20	13

The age group between 50 -69 years were affected more (39 %) followed by 30 -49 years (34%),18–29years 14% and more than 70 years 13%,Percentage of Males involved in this study: 57.8: Percentage of Females involved in this study 42.2.

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Figure 1: Distribution of Serum glucose in various Age group

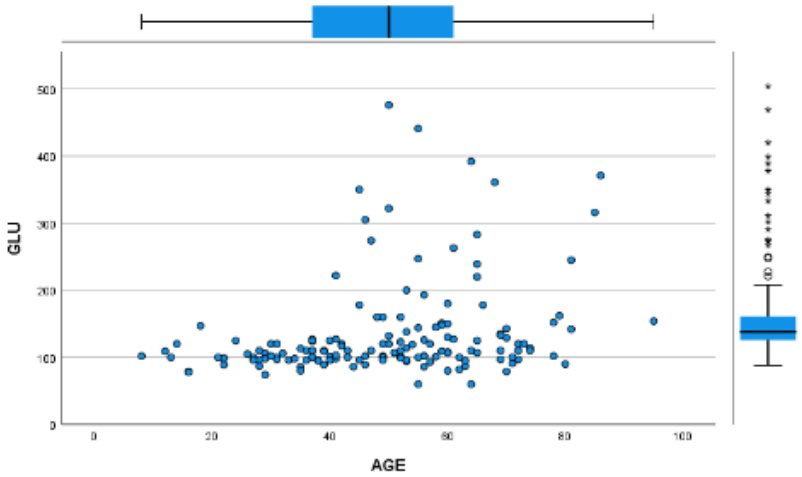

Figure 2: Distribution of Serum C- Reactive Protein in various Age group and sex

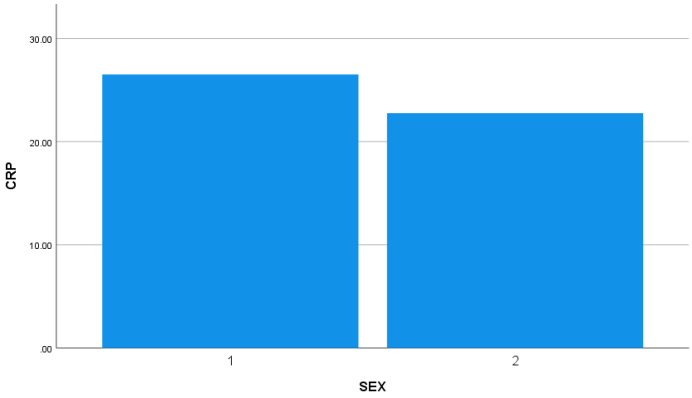

Table 2: Chi-square test:

	AGE	SEX	ESR	GLU	CRP	D-dimer	LDH	Ferritin
Chi-Square	57.948[a]	3.740[b]	67.506[c]	199.000[d]	306.299[e]	93.351[f]	46.909[g]	64.571[h]
df	63	1	51	76	66	106	118	98
Asymp. Sig.	0.656	0.053	0.061	0.001	0.001	0.805	1	0.996

Table 3: Mean and Standard deviation of various Biochemical and haematological parameters of Covid Patients involved in our Study

	Mean	Std. Deviation(+-)	Std. Error Mean

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Age	49.48	17.19	1.38
Neutrophil count	69.57	14.22	1.15
WBC	7440.06	3298.73	265.82
Glucose	134.28	71.77	5.78
Urea	37.9	26.42	2.13
Creatinine	1.14	0.83	0.07
ESR	43.97	27.57	2.22
Lymphocyte count	24.4	13.55	1.09
CRP	24.93	41.35	3.33
LDH	313.27	213.46	17.20
D-dimer	427.13	526.5	42.42
Ferritin	300.23	303.07	24.42
Sodium	140.59	5.94	0.84
Potassium	5.204	6.22	0.88
APTT	33.69	12.08	1.71
PT	11.64	1.59	0.22

Table 4: Pearson correlation of Age and Sex with haematological markers in Covid -19 patients

		SEX	*NEUTRO*	*LYMPHO*	*CRP*	*D-dimer*	*Ferritin*	*AGE*
SEX	*Pearson Correlation*	*1*	*0.079*	*-0.077*	*-0.045*	*-0.027*	*-0.152*	*-0.113*
	Sig. (2-tailed)		0.332	0.342	0.580	0.742	0.061	0.163
NEUTRO	Pearson Correlation	0.079	1	-.963**	.230**	-0.136	0.152	.256**
	Sig. (2-tailed)	0.332		0.000	0.004	0.093	0.060	0.001
LYMPHO	Pearson Correlation	-0.077	-.963**	1	-.219**	0.155	-0.143	-.215**
	Sig. (2-tailed)	0.342	0.000		0.006	0.055	0.076	0.007
CRP	Pearson Correlation	-0.045	.230**	-.219**	1	0.091	0.040	0.120
	Sig. (2-tailed)	0.580	0.004	0.006		0.263	0.623	0.138

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D-dimer	Pearson Correlation	-0.027	-0.136	0.155	0.091	1	0.025	-0.117
	Sig. (2-tailed)	0.742	0.093	0.055	0.263		0.755	0.147
Ferritin	Pearson Correlation	-0.152	0.152	-0.143	0.040	0.025	1	.167*
	Sig. (2-tailed)	0.061	0.060	0.076	0.623	0.755		0.039
AGE	Pearson Correlation	-0.113	.256**	-.215**	0.120	-0.117	.167*	1
	Sig. (2-tailed)	0.163	0.001	0.007	0.138	0.147	0.039	

Table 5: Spearman's correlation of haematological and biochemical markers in covid-19 patients

		WBC	NEUTRO	LYMPHO	D-dimer	Ferritin	PT	APTT	GLU	UREA	CREA
WBC	Correlation Coefficient	1.000	.347**	-.361**	0.079	.222**	0.225	0.265	.165*	0.151	.211**
	Sig. (2-tailed)		0.000	0.000	0.333	0.006	0.117	0.063	0.041	0.062	0.008
NEUTRO	Correlation Coefficient	.347**	1.000	-.962**	0.082	0.145	0.103	0.003	.169*	0.055	.184*
	Sig. (2-tailed)	0.000		0.000	0.314	0.073	0.476	0.983	0.037	0.497	0.022
LYMPHO	Correlation Coefficient	.361**	.962**	1.000	0.100	-0.142	0.121	0.022	0.129	0.060	-.172*
	Sig. (2-tailed)	0.000	0.000		0.218	0.079	0.401	0.880	0.112	0.463	0.033
D-dimer	Correlation Coefficient	0.079	-0.082	0.100	1.000	0.037	0.100	.467**	0.020	0.083	-.162*
	Sig. (2-tailed)	0.333	0.314	0.218		0.644	0.491	0.001	0.808	0.308	0.045
Ferritin	Correlatio	.222**	0.145	-0.142	0.037	1.000	0.181	0.007	0.117	0.091	.196*

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	n Coefficient										
	Sig. (2-tailed)	0.006	0.073	0.079	0.644		0.209	0.961	0.149	0.262	0.015
PT	Correlation Coefficient	0.225	-0.103	0.121	0.100	0.181	1.000	0.076	0.044	0.016	0.106
	Sig. (2-tailed)	0.117	0.476	0.401	0.491	0.209		0.598	0.760	0.915	0.462
APTT	Correlation Coefficient	0.265	0.003	-0.022	.467**	0.007	0.076	1.000	0.086	0.052	0.098
	Sig. (2-tailed)	0.063	0.983	0.880	0.001	0.961	0.598		0.554	0.720	0.498
GLU	Correlation Coefficient	.165*	.169*	-0.129	0.020	0.117	0.044	0.086	1.000	.429**	.400**
	Sig. (2-tailed)	0.041	0.037	0.112	0.808	0.149	0.760	0.554		0.000	0.000
UREA	Correlation Coefficient	0.151	0.055	-0.060	0.083	0.091	0.016	0.052	.429**	1.000	.526**
	Sig. (2-tailed)	0.062	0.497	0.463	0.308	0.262	0.915	0.720	0.000		0.000
CREA	Correlation Coefficient	.211**	.184*	-.172*	-.162*	.196*	0.106	0.098	.400**	.526**	1.000
	Sig. (2-tailed)	0.008	0.022	0.033	0.045	0.015	0.462	0.498	0.000	0.000	

Table 6: Pearson correlation of inflammatory markers with Liver Function Tests in Covid-19 patients

		ESR	CRP	Ferritin	T.BIL	D.BIL	IN.BIL	AST	ALT	ALP	T.pro	Alb	Globulin
ESR	Pearson Correlation	1	.508**	0.083	0.044	0.019	0.064	0.183	0.123	0.062	0.121	0.108	0.087

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	Sig. (2-tailed)		0.000	0.305	0.763	0.896	0.658	0.209	0.401	0.674	0.407	0.461	0.552
CRP	Pearson Correlation	.508	1	0.040	0.167	0.279	0.230	0.088	0.141	0.015	0.177	-0.077	-0.199
	Sig. (2-tailed)	0.000		0.623	0.246	0.052	0.108	0.546	0.333	0.918	0.225	0.600	0.171
Ferritin	Pearson Correlation	0.083	0.040	1	0.000	0.080	0.112	0.028	0.186	0.197	0.007	-0.067	0.049
	Sig. (2-tailed)	0.305	0.623		0.999	0.586	0.437	0.847	0.200	0.174	0.961	0.648	0.737
T.BIL	Pearson Correlation	0.044	0.167	0.000	1	0.948	0.371	0.120	0.082	0.050	0.111	0.049	-0.212
	Sig. (2-tailed)	0.763	0.246	0.999		0.000	0.008	0.410	0.575	0.735	0.448	0.737	0.143
D.BIL	Pearson Correlation	0.019	0.279	-0.080	.948	1	.291*	0.177	0.034	0.062	0.177	0.083	.357*
	Sig. (2-tailed)	0.896	0.052	0.586	0.000		0.043	0.230	0.816	0.674	0.228	0.573	0.013
IN.BIL	Pearson Correlation	0.064	0.230	-0.112	0.37	.291*	1	0.057	0.078	.385	.340*	0.161	0.371**
	Sig. (2-tailed)	0.658	0.108	0.437	0.008	0.043		0.699	0.594	0.006	0.017	0.269	0.009
AST	Pearson Correlation	0.183	0.088	0.028	0.120	0.177	0.057	1	.665	0.118	0.181	-0.216	-0.080
	Sig. (2-tailed)	0.209	0.546	0.847	0.410	0.230	0.699		0.000	0.419	0.213	0.136	0.583
ALT	Pearson Correlation	0.123	0.141	-0.186	0.082	0.034	0.078	.665**	1	0.102	0.128	-0.188	-0.024
	Sig. (2-tailed)	0.401	0.333	0.200	0.575	0.816	0.594	0.000		0.484	0.382	0.195	0.868
ALP	Pearson Correlation	0.062	0.015	-0.197	0.050	0.062	.385**	0.118	0.102	1	.297*	-0.262	-0.215
	Sig. (2-	0.67	0.91	0.174	0.73	0.67	0.006	0.41	0.48		0.03	0.06	0.138

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	tailed)	4	8		5	4		9	4		8	9	
T.pro	Pearson Correlation	0.121	0.177	-0.007	0.111	0.177	-.340*	0.181	0.128	.297*	1	.773**	.822**
	Sig. (2-tailed)	0.407	0.225	0.961	0.448	0.228	0.017	0.213	0.382	0.038		0.000	0.000
Alb	Pearson Correlation	0.108	0.077	-0.067	0.049	0.083	0.161	0.216	0.188	0.262	.773**	1	0.275
	Sig. (2-tailed)	0.461	0.600	0.648	0.737	0.573	0.269	0.136	0.195	0.069	0.000		0.056
Globulin	Pearson Correlation	0.087	0.199	0.049	0.212	.357*	.371**	0.080	0.024	0.215	.822**	0.275	1
	Sig. (2-tailed)	0.552	0.171	0.737	0.143	0.013	0.009	0.583	0.868	0.138	0.000	0.056	

Discussion:

COVID-19 constitutes a major public health problem at world level and is related with multiple complications that affect both the health of patients and the socio- economic status of the patient. With the continuous spread of COVID-19 infection across the world and different theories of its effect on the human body are also flashing every day, we are still inexperienced in understanding a few aspects of COVID-19. However, we still have a lot to know about the effect of COVID-19 on different hematological and biochemical profiles in COVID-19 patients. Therefore, we analyzed the hematological and biochemical characteristics of 154 patients and correlated each other, this correlation is useful to the clinicians for early diagnosis and management according to laboratory values.

The severity of HCo-V induced upper respiratory tract infection ranged from mild to severe, depending upon the individual's age. And also, those people who were already having lung and cardiac problems exhibited severe infectious conditions. The SARS-CoV, MERS-CoV, and SARS-CoV-2, are found to be deadly pathogenic, causing ARDS, hepatic diseases, intestinal diseases, multiple organ failure, and eventually, may lead to death in severe cases [9, 10,11]. The older population had more number of comorbidities, limited organ function, reduced lung capacity, impaired immune system, biological aging, and more severe complications, these are the common reason pointed in earlier research on elderly with COVID-19. [12, 13]

Although predisposing factors had been defined (gender, risk ages and association with pathologies such as obesity, diabetes and hypertension), it is ultimate to use biomarkers that would allow a distinction to be made in concerning the progression of the disease and to decide the management for patients. The available evidence showede that patients with severe and critical cases presented with various alterations in their laboratory parameters, and in some cases rapidly progressed toward ARDS and septic shock followed by multiple organ failure [14,15]. In some studies Kidney function biomarker such as serum creatinine were in general observed to be within the reference range and no statistically significant differences were found between the mean values of patients with mild and severe conditions on admission. In our study abnormal renal function tests were noted with increase in age. During the time of hospitalization, however, it was observed that the non-surviving patients had a progressive increase (approximately as of day 10 after admission) above the reference range, reached a peak few days before they died. Therefore, biomarkers could be used to assess the prognosis of patients in the course of their

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hospitalization. Renal dysfunction in Mild COVID-19 cases was minimal and was not diagnosed as acute kidney injury[16, 17, 18].

In our study, males were affected more than females; the age group between 50 -69 years were affected more 39 %. Age and sex of the study population had been correlated with various biochemical and haematological parameters. Acute kidney injury was noted as a complication of covid infection. Serum urea had significant correlation with age, LDH and ferritin. Serum globulin positively correlated with bilirubin ($p < 0.01$). Increased neutrophil counts were noted alongside of elevated ESR, CRP and LDH. Studies by Ruan et al and Xiang et al showed similar results [19,20]. Similarly, in our study also inflammatory markers were elevated according to disease severity.

In Covid patients, hyperglycaemia was common in age more than 40 years which also implied covid severity is related to diabetes; in our study hyperglycaemia was predominant in females than in males (Figure 1). ESR elevation was noted in all age groups and was not specific to age and sex. Total WBC count < 5000 was noted in 24 % of covid-19 patients, 50000 -11000 in 67% and more than 11000 in 9% patients. CRP was elevated in age group of more than 30 years and in males (Figure 2). In our study, D –dimer had positive correlation with lymphocyte count, serum urea, ESR and aPTT. Likewise, ferritin had positive correlation with total count, neutrophil count and lymphocyte count. The liver function tests were not correlating significantly with disease severity. Similar results were noted in studies by Zhou et al, Velavan and Liu et al. [21, 22, 23].

Conclusion

According to our study, laboratory investigations play an important role in the SARS-CoV-2 pandemic, not only in a diagnostic point of view but also in terms of the prognosis and management of COVID-19 patients. Likewise, the laboratory work allows optimizing the hospital environment resources of the critical units of the health systems, resulting in the enhancement of the response time and its efficiency. This study concludes that serum Glucose, Ferritin, CRP, D-dimer, Bilirubin, Globulin and LDH play an important and independent role in early diagnosis and prognosis in Covid 19 infection.

Ethical Statement

This study was approved by the Institutional Ethical Committee of the Government Tirunelveli Medical College and Hospital, Tirunelveli, Tamil Nadu, India.

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