Correlation of Hematological and Immunological Parameters with Disease Severity and Outcome in COVID-19 Patients

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Abstract- Corona Virus disease has shown wide-ranging clinical signs, the spectrum of clinical symptoms ranges from mild flu to pneumonia-like symptoms which can evolve into acute respiratory distress syndrome (ARDS). To give an idea about the diagnosis and hyper inflammation condition, a collection of laboratory tests was carried out. The objective of this study was to investigate the inflammatory and hematological parameters of COVID-19-positive individuals and to correlate these parameters with disease severity. A comparative cross-sectional study was conducted in CMH Lahore for a six-month duration, on a sample size of 120 individuals. Patients were divided into three groups: mild, moderate, and critical; on the basis of their clinical condition. The hematological and immunological biochemical parameters comprising WBC count, platelets, serum d-dimers, and C-reactive proteins were examined in the pathology laboratory at CMH Lahore Hospital. The data was analyzed using SPSS version 25 and Graph-pad Prism. Total Leukocyte count levels were raised in recovered critical patients (p value ≤ 0.05) as well as in dead patients (p value ≤ 0.05) as compared to recovered mild patients and recovered moderate patients. Dead patients had shown significantly higher levels of CRP (237.3 mg/L). d-Dimers levels were significantly correlated with the severity of disease in all three groups of dead patients ($r=0.57$ $p\leq 0.05$). No significant difference was observed in the number of platelets in the study groups. Investigated hematological and immunological parameters were correlated with disease severity and mortality. These parameters were significantly raised in critically diseased patients as compared to mild and moderately diseased patients except for the platelet counts.

Index Terms- : COVID-19; disease severity; hematological parameters; Immunological Parameters; outcome

I. Introduction

A group of viruses known as coronavirus is responsible for human respiratory illnesses. The novel coronavirus strain (SARS-CoV-2) was initially discovered in Wuhan, a Chinese city, in December 2019.[1,2] On March 12, 2020, the World Health Organization designated COVID-19 a global pandemic that was spreading across the globe. WHO has reported a total of 229 countries affected around the world with approximately 678,582,108 confirmed cases and 6 million mortalities globally.[3]

Corona Virus disease has shown wide-ranging clinical signs, the spectrum of clinical symptoms ranges from mild flu to pneumonia-like symptoms which can result in the development of ARDS, which can result in mortality.[4] Universal symptoms are dry cough, fever, and fatigue. The most frequent radiologic findings on chest computed tomography (CT) of diagnosed COVID-19 individuals are ground glass opacity and consolidation lesions.[5] To give an idea about the diagnosis and hyper inflammation condition, a collection of laboratory tests have been carried out including lymphocyte count, white blood cells count, platelets count, neutrophil count, etc.[6] Noticeable lymphopenia is being seen in the acute phase of the disease including excessive loss of T helper cells.[7] A variety of laboratory features for example lymphopenia elevated D-dimer, and C-Reactive Protein (CRP) is linked to COVID-19 disease's severity.[8] Myriad studies on Corona Virus Infection have inspected the relation between irregularities of laboratory measures with the severity as well as mortality of the disease.[9]

This study, probed the leucocyte number, platelets count, C-reactive proteins, and D-dimers of individuals with established COVID-19 disease and a classic clinical outcome (death or discharge) of the individuals being admitted to CMH Lahore Hospital.

The purpose of this study was to assess inflammatory and hematological parameters of COVID-19-positive individuals and to relate disease severity through the outcome of inflammatory and hematological parameters.

II. METHODOLOGY

With convenient sampling a comparative cross-sectional study was conducted in CMH Lahore for a duration of 6 months. World Health Organization (WHO) calculator with the following formula was applied for sample size calculation.

$$n = 2\sigma^2 \frac{(z_{1-\alpha/2} + z_{1-\beta})^2}{(\mu_1 - \mu_2)^2}$$

Where:

μ_1 was the anticipated mean in the population

μ_2 was the anticipated mean in cases.

Z 1- β was the desired power of study = 95

Z 1- α /2 was desired level of significance = 0.05

n was the calculated sample size =120

The patient who tested negative for COVID-19 disease and also the patients with comorbid conditions were excluded from the study. The patient who tested positive for COVID-19 disease were included in this study.

Out of the total sample size of 120 individuals, 85 were recovered patients and 35 were dead patients. Upon severity of the disease, all the subjects were then further divided into three groups depending on Oxygen saturation and high resolution computed tomography into mild, moderate and severe.

Mild: COVID-19 positive with slight clinical manifestations but no significant radiological findings on imaging.

Moderate: COVID-19 positive with respiratory tract inflammatory symptom and classical radiological findings of pneumonia.

Severe: COVID-19 positive with severe respiratory symptoms, Oxygen saturation < 94% on room air, respiratory rate >30breaths /min, and lung infiltrate >50%.

Sample Processing Procedure: The nasopharyngeal swab was taken for RT-PCR whereas venous blood was collected from each individual and analyzed for inflammatory and hematological parameters. About 3ml of blood from the cephalic vein of each participant was collected at admission. The hematological and immunological biochemical parameters comprising WBC count, platelets, serum d-dimers, C-reactive proteins, and other indexes were examined by the Pathology laboratory at CMH Lahore Hospital

Study Protocol and Ethical Consideration: The study was conducted in accordance with the declaration and approval of the Ethics Committee of CMH, Lahore Medical College & IOD, Ethical Review Board (ERB). Confidentiality and privacy of participants was maintained as no personal information (name, contact number) was asked. Furthermore, information obtained from the survey was only used for the purpose of research. A predesigned patient performa containing demographic information, detailed medical history, and laboratory findings of patients was filled with informed consent after explaining the purpose of this study. In case of lack of any detail, the doctors or other healthcare workers who were in charge were consulted on the spot.

Statistical Analysis: SPSS (Statistical Program for Social Sciences) version 25 and Graph-Pad Prism were used to organize and analyze the data. Data were evaluated for its normality by the Shapiro-Wilk test. Mean ± SD was applied for symmetrical data. ANOVA test was put into application for a comparison of the mean between three groups whereas, in order to determine the correlation between disease severity and its outcome, we applied Pearson's correlation.

III. RESULTS

Demographic details of study participants: The descriptive statistics of all the COVOD-19 patients are listed in **Table 1**. The mean age of the recovered patients was 43.49±11.3 years whereas the patients who expired had a mean age of 51.88±10.7, were the dead patients. The overall average age was 45.94±11.3 whereas the mean age for critical patients was 54.2±8.88 years.

Table 1: Age Distribution in COVID-19 patients.

Age Distribution		Mean (years)	Standard deviation
Total population		46.6167	11.31607
Recovered patients (n=85)	**total recovered patients (n=85)**	43.4941	10.75221
	Mild (n=20)	41.6500	11.63151
	Moderate (n=30)	39.3000	9.99017
	Severe (n=35)	48.1429	9.22638
Deceased patients (n=35)	**Total deceased patients (n=35)**	54.2000	8.88753
	Mild (n=8)	54.2500	9.63253
	Moderate (n=10)	56.4000	8.99630
	Severe (n=17)	52.88	8.76

Hematologic Abnormalities in COVID-19 patient

TLC, Platelet count, CRP and D-dimers were the hematological parameters studied in the present article. The comparative study showed that all of these parameters had a statistically strong significant p-value. (Table 2)

Table 2 comparison of hematological parameters among recovered and deceased patients.

Parameters	Recovered patients	Deceased patients	p-value*
Total leucocyte count $x10^9$/L ($5\text{-}11\times10^9$/L)	12.56+3.71	17.67+6.08	<0.01
Platelets count $x10^9$/L ($156\text{-}342\times10^9$/L)	245.45+79.93	300.49+84.26	<0.01
CRP, mg/L (>6mg/L)	76.88+80.42	198.45+100.72	<0.01
D-dimers, ng/mL (<250ng/mL)	518.83+309.13	824.82+546.25	<0.01

***p-value was calculated using independent sample T test.**

These parameters were further compared among the groups based on severity of the disease in both recovered and deceased patients and were found to be indicators of adverse outcomes. The results of the comparative study between the groups and within the groups for recovered and dead patients are given in **Table 3 and Table 4**. The Post Hoc analysis all of the groups of recovered and deceased found a significant difference in their means of TLC. Although no significant difference was noted in any of the groups among recovered for platelet count and CRP, but D-dimers were significantly raised among severe subjects

with both mild and moderate patients. In the dead subjects there was a significant difference between multiple groups for platelet count, CRP and D-dimers as clear from the figure 1.

Table 3. Group wise comparison of laboratory markers in recovered patients.

Variable	Study Groups Mean ±SD			Anova	*p*-value*	Post hoc test
	Mild (n=20)	Moderate (n=30)	Severe (n=35)			
Total leucocyte count x10^9/L	8.9 ± 3.9	12.2 ± 2.3	14.91 ± 2.70	27.174	≤0.01	(Mild and moderate) (Mild and severe), (Moderate and severe)
Platelets count x10^9/L	225.7 ± 84.9	244.7 ± 79.6	257.3 ± 77.4	0.997	0.373	_____
CRP, mg/L	71.1 ± 82.8	78.7 ± 82.6	78.6 ± 79.4	0.065	0.937	_____
D-dimers ng/mL	361.5 ± 166	380.7 ± 143.8	727.1 ± 356.4	19.488	≤0.01	(Mild and severe), (Moderate and severe)

*p-value was calculated after apply one way ANOVA.

Table 4. Group wise comparison of laboratory markers in deceased patients.

Variable	Study Groups Mean ±SD			Anova	*p*-value*	Post hoc test
	Mild (n=20)	Moderate (n=10)	Severe (n=20)			
Total leucocyte count x10^9/L **(5-11×10^9/L)**	10.8 ± 2.6	15.8 ± 2.9	22.1 ± 4.9	22.750	≤0.01	(Mild and moderate) (Mild and severe), (Moderate and severe)
Platelets count x10^9/L **(156-342 ×10^9/L)**	245.9 ± 72.2	305.7 ± 71.5	323.1 ± 88.8	2.519	0.096	(Mild and severe),
CRP, mg/L **(>6mg/L)**	93.6 ± 49.8	216.2 ± 106.7	237.4 ± 82.3	8.192	≤0.01	(Mild and moderate) (Mild and severe),
D-dimers, ng/mL **(<250ng/mL)**	480.9 ± 191.4	510.2 ± 181.3	1171.8 ± 592.1	10.340	≤0.01	(Mild and severe), (Moderate and severe)

*p-value was calculated after apply one way ANOVA

Association between COVID-19 patients' outcome and various parameters: Pearson correlation test was used for correlating variables with the severity of the disease in dead patients of COVID-19. The relationship among the parameters of our study participants at different severity levels of COVID-19 dead patients is shown in Table 5. There is a strong positive correlation between all these parameters with disease severity in COVID-19 dead patients. Briefly, a strong correlation with

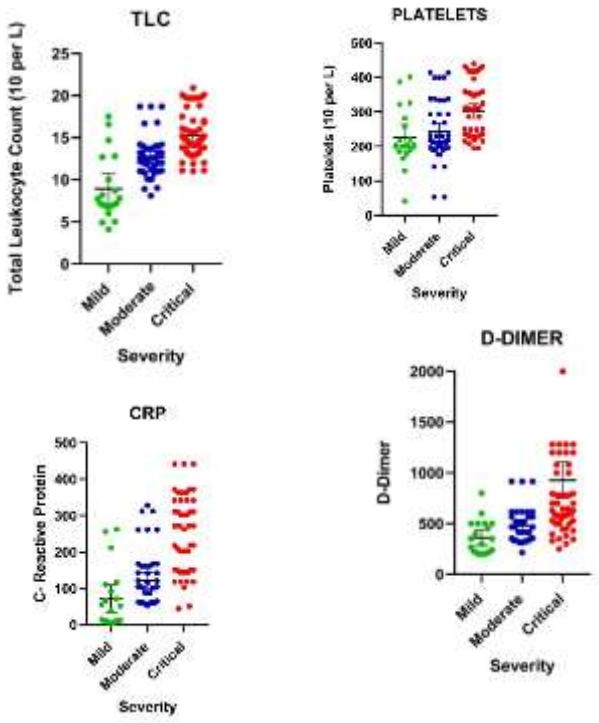

Figure 1. ANOVA and Multiple Comparisons between all stages of the disease in dead patients.

severity of disease had been noticed in TLC levels (r = 0.765 p≤0.01). Similarly, D-dimers and CRP have a positive correlation with severity (r=0.57 p≤0.01, r=0.536 p≤0.01) respectively. However, platelet count has shown a significant positive relation with disease severity in our study participants (r=0.351, p≤0.05) which contradicted the results of various studies done previously (mostly showing a negative correlation), in order to predict the correlation between platelets count and disease severity.

Table 5: Pearson correlation between studied parameters and COVID-19 disease severity for dead patients.

Parameters	**Severity**	
	Rho	***p*-value**
Total Leukocyte count	.765**	.000
Platelets	.351*	.039
C-reactive protein	.536**	.001
D-dimers	.570**	.000

* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

IV. DISCUSSION

Since the emergence of coronavirus disease in the year 2019, it has always been imposing a burden on the healthcare system with its emerging strains every now and then. Despite, the proper documentation of the epidemiological and clinical characteristics of the patients, the clinical spectrum of Covid-19 patients is yet to be understood.[10] In recent times, biomarkers of systemic inflammation which are now part of expanded CBC tests, are

being investigated by clinicians because they are easy to perform and are also low on costs. Conferring to studies conducted with COVID-19, the inflammatory and hematological parameters are useful prognostic markers.[11,12]

The current study was conducted on COVID-19-positive patients. The total sample size was further divided into mild, moderate, and severe patients based on their levels of oxygen saturation at room temperature and radiological findings of lung HRCT. Total leukocyte count, platelet counts, C-reactive protein, and D-dimers are the inflammatory and hematologic markers being investigated in this study. Through detailed analysis of the clinical data of these patients, we had found a significant correlation between the severity of disease and outcomes in patients with these deranged biomarkers levels.[13]

An essential blood measure for determining the disease's severity is the total leukocyte count. According to this study, the levels of total leukocyte count are on the rise as the condition worsens. In a comparison of the patients with elevated total leukocyte count and with non-elevated total leukocyte count, the patients with elevated total leukocytes counts were more likely to develop severe illness and end-organ damage This can be seen in concurrence with a study being conducted in Tehran city of Iran, where they reported that, a lower lymphocyte count and a greater total leukocyte count, associated with COVID-19 disease severity and reflects a poorer clinical outcome.[14] An analogous study conducted on 619 confirmed COVID-19 patients, in Wuhan city of China, noted an increase in total leukocyte count in older patients with underlying comorbidities.[15]

Several investigations have revealed a connection between thrombocytopenia and COVID-19 patients' disease severity. It is remarkable to note that, our study has not found a significant association between mean platelet count and disease severity in all the subgroups of dead patients, similarly, we have not find any correlation between disease severity and platelet count in recovered patients. This observation has further been substantiated by a retrospective cohort study conducted in Turkey, which has shown comparable results, with no statistically significant relationship between platelet levels among survivors and non-survivors.[16] Neslihan Ozcelik found different results in his study, while comparing the levels of mean platelet count in the COVID-19 group and influenza group, there were statistically significantly lower levels of Mean platelet count in the COVID-19 group.[17]

CRP is regarded as a standalone measure that aids in the early diagnosis of COVID-19 disease severity and provides information on the prognosis of the condition. It has been seen through numerous studies that higher rates of CRP are being reported in severe COVID-19 patients. Moreover, related results are observed in our study, where levels of CRP are found to be significantly raised in severe COVID-19 patients. Whereas this result was not consistent for the recovered patients according to our study. A study conducted in Morocco has concluded CPR to be a robust predictor of adverse outcomes and an independent discriminator of severe/critical illness in comparison to other biological markers.[18] Similarly, research conducted in Dhaka city of Bangladesh summarized their results by declaring CRP as a good predicting performer in COVID-19 severity as evaluated by ROC analysis.[19] These findings are further substantiated by a study conducted at a national level, which studied the association of plasma CRP with disease severity and achieved similar results.[20]

The D-dimer test is the forecaster of the development of thrombosis in COVID-19 disease patients and its prognosis as well. Unfortunately, higher levels of D-dimer in patients with COVID-19 are usually linked to a poor prognosis.[21] Investigations being done in this study suggested comparable results. By comparing groups of recovered patients to patients in the dead group, we discovered a much higher association between the D-dimer test values in the deceased group of patients. Research being conducted on 1561 patients in Tongji Hospital, Wuhan city of China, high lightened the fact that the laboratory reports of severe patients exhibited raised levels of D-dimers and a significant correlation between disease severity and consequences in critically ill COVID-19 patients.[22] This evidence was substantiated by another study conducted retrospectively in COVID-19 hospitalized patients in New York City, America. D-dimer levels were recorded within 3 days of admission to the hospital followed by an assessment of at least 3 D-dimer levels previous to the outcome of interest. The risks of mortality, the need for mechanical ventilation, and venous thromboembolism were all linked to higher D-dimer levels (VTE). Disagreement was raised by the finding that D-dimers alone did not appear to be a reliable indicator of COVID-19 patient outcomes.[23]

Our study has numerous limitations, particularly because of a lesser sample size. Secondly, the subjects in this study were mainly from a single city and country so, the results of this study cannot be deduced from other racial groups. Furthermore, only hospital-admitted patients are studied, outpatients and their clinical features were not considered in our study. Assessment of this topic should be done to provide the finest supervision to the patient during the COVID-19 pandemic. Quite a lot of other blood parameters must be studied for the assessment of the severity and impermanence of the COVID-19 pandemic.

V. CONCLUSION

Hematological and immunological parameters such as total leukocyte count, platelet count, C-Reactive Proteins, and D-dimers are correlated with disease severity and mortality. These parameters are significantly raised in critically diseased patients as compared to mild and moderately diseased patients except for the platelets count according to our study.

VI. ACKNOWLEDGMENT

The authors are thankful to CMH Lahore hospital for supporting this study.

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