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Relationship between Inflammatory Markers and Disease Outcome in COVID-19

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ABSTRACT

Background: Coronavirus disease-2019 (COVID-19) has been labelled as a global pandemic and a cause of documented high mortality rates among severe or critical patients. This has been linked with hyperinflammation of the innate and adaptive immune systems and the resulting cytokine storm. Our study aims to evaluate the relationship between inflammatory markers and disease outcomes in COVID-19.

Methodology: This cross-sectional study was conducted at Federal Government Polyclinic (FGPC) Hospital, Islamabad for a period of 3 months. All the patients who had either RT-PCR for COVID-19 or HRCT findings suggestive of COVID were included in the study. The inflammatory markers like C-reactive protein (CRP), D-Dimers, and Lactate dehydrogenase (LDH) were done and a combined score DLC was calculated. The need for Oxygen (litres/min), Bilevel positive airway pressure (BIPAP), or Mechanical Invasive Ventilation was documented.

Results: The study was conducted on 213 patients which included 119(56%) male patients and 94(44%) female patients. COVID-19 PCR was positive in 193(90.6%) while 20 patients (9.4%) had a negative PCR but HRCT suggestive of COVID. HRCT was done in a total of 46 patients out of the 213 patients and 45 had features suggestive of COVID-19. The AUROC was 0.686(0.61-0.75) for D dimers, 0.688(0.61-0.75) for LDH, and 0.649(0.517-0.72) for CRP respectively, and Spearman rho values of (0.326, 0.328 and 0.266) respectively with a p-value (0.000).

Conclusion: D-dimer, LDH and CRP individually as well as a combined score of the inflammatory markers (DLC) and Oxygen requirement had a significant correlation with the mortality of the COVID-19 patients.

Key words: COVID-19, CRP, LDH, D dimers, DLC score.

Authors' Contribution:
[1,2]Conception; Literature research; manuscript design and drafting; [2,3] Critical analysis and manuscript review; [5,6] Data analysis; Manuscript Editing.

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Introduction

Ever since the early days of the human era, a great number of deadly infectious diseases have affected people and illnesses like malaria, tuberculosis, influenza, and smallpox have killed thousands of patients over time. The Infectious disease of the 21st century, caused by Novel Coronavirus or SARS-CoV-2 (labelled COVID-19) began in Wuhan, China in December 2019 and quickly spread like wildfire to all continents. Ultimately, World Health Organization (WHO) declared it a global pandemic on March 11,

2020.[1] Till December 2022, there have been approximately 651million confirmed cases with more than 6 million deaths, reported to WHO. [2] At present, it seems to be impracticable to curtail the disease spread, and the focus is directed towards better treatment and identification of factors that are associated with severity of the disease.[3] Covid-19 is a highly infectious disease with variable presentation ranging from mild to critically ill patients.[4,5] The symptoms vary from asymptomatic, low-grade pyrexia, dry cough, sore throat, breathlessness, tiredness, body aches, fatigue, myalgia, nausea, vomiting, and diarrhea, to severe bilateral consolidation pneumonia, acute respiratory distress syndrome (ARDS) and multiple organ impairment leading to death.[6,7] Collective evidence has suggested that inflammatory response plays a crucial role in the progression of COVID-19.[8] The intense viral replication of SARS-CoV-2 and cellular destruction cause macrophages, monocyte recruitment leading to the release of cytokines, and chemokines resulting in a cytokine storm.[9] It has been postulated by several studies that raised levels of inflammatory markers such as high C-reactive protein (CRP) levels, interleukin (IL)-2, IL6, IL-10, and tumor necrosis factor (TNF) is directly proportional to worse outcome.[6] Reduced lymphocyte count and elevated levels of ferritin, LDH, and D-dimer were also reported to be associated with disease severity.[10,11] However, the relationship between laboratory parameters and disease severity as well as risk- stratification is still not well defined. The objective of this study was to understand the relationship between inflammatory markers and COVID-19 and also introduce a new scoring system i.e. the DLC score for the severity of COVID-19. More specifically, the study assesses their implications in determining disease progression

Methodology

This Retrospective study was conducted at Federal Government Polyclinic (FGPC) Hospital, Islamabad for a period of 3 months. The study was approved by the Institutional Ethical Review Board. The record of patients with COVID-19 from January 2020 onwards was analysed. All the patients who had clinical features, positive PCR for COVID-19, or High-Resolution Computed Tomography (HRCT) findings suggestive of COVID-19 were included in the study. Patients who had seasonal allergies, incomplete clinical records, neither HRCT findings nor a positive COVID-19 PCR, and had other systemic infections like enteric fever, malaria, and dengue fever were excluded from the study. The sample size was calculated using the WHO calculator by taking the total number of cases of covid 19 till date in Islamabad which were 139844 for a total of 1.198 million (11.6%) population.[12] Keeping the confidence interval at 95% and margin of error at 5% the sample size came out to be 158. In total 213 patients were included in this study. The consecutive non-probability sampling technique was used to collect the data. After taking the demographic details of patients and defining the diagnostic criteria, which was either positive PCR for COVID-19 or HRCT chest findings suggestive of COVID-19, all inflammatory markers which have been done for the patients were recorded that included, C- Reactive Protein (CRP), D dimers, and Lactate Dehydrogenase (LDH) levels. The number of patients who required oxygen was also noted and the different categories depending on the amount of oxygen required were also identified with the further need for either Bi-level Positive Airway Pressure Ventilation (BIPAP) or Invasive Mechanical Ventilation. The final outcome of the patient in term of morbidity or mortality was recorded.

COVID-19 PCR was done using the standard method of taking nasopharyngeal swabs. HRCT was done only in the patients who could be mobilized from the hospital to a nearby facility and those who had a negative PCR but had Chest X-Ray (CXR) findings suggestive of COVID-19. Blood samples like LDH, CRP, and D dimers were done on admission and thereafter serially, to see the degree of

inflammation. A new combined scoring system of three commonly used inflammatory markers i.e. D-Dimers, LDH, and CRP, named DLC score was used to find its association with the oxygen requirement. Oxygen requirement was titrated as per the severity of the patient and the degree of lung damage. The patient was put on either BIPAP or Mechanical Invasive Ventilation when the oxygen requirement exceeded 15 litres/min.

The data were analysed using SPSS version 23. The frequencies and percentages for the qualitative variables like gender, oxygen dependency, the need for BIPAP or mechanical ventilation, and the positive or negative COVID -PCR and HRCT findings were calculated. Mean, Standard deviations, minimum and maximum values for the quantitative variable like D dimers, CRP, LDH, and the combined DLC score were calculated. and the intergroup comparison for the different inflammatory markers was done. The correlation between the different inflammatory markers and mortality was found using the chi-square test. The Receiver Operating Characteristic (ROC) curve analysis was carried out to determine the sensitivity and specificity of these three inflammatory markers. The combined DLC score with a cut-off value of 758 was calculated by adding the upper limits of normal values for each of the individual variables i.e LDH <248, IU/L, D Dimers< 500mg/l and CRP of <10 mg/l. The impact of the DLC score on the oxygen requirement and the final outcome i.e mortality was also assessed.

Results

This study was conducted on 213 patients which included 119(56%) male patients and 94(44%) female patients. Covid-19 PCR was positive in 193(90.6%) while 20 (9.4%) had a negative PCR (with findings suggestive of COVID-19 on HRCT). HRCT was done in a total of 46 patients, among these 45 had features suggestive of COVID-19. Two hundred and eight (97.7%) were oxygen dependent while 5 (2.3%) did not need oxygen. Of the 213 patients, 49 patients needed BIPAP (23%) while 24 patients needed invasive ventilation. The overall mortality was 46.9% (100/213).

The baseline characteristics are mentioned in Table 1. Mean age of the patients was 59.9±15.71 years. D-Dimer (2856.22±3675.25), LDH (506.92± 321.44), CRP (101.66 ± 56.53), and DLC scores (3468.11±3750.70) were reported in order to find the difference in the values in relation to patient outcomes as given in Table 2.

The DLC score (which is a sum of the D dimers, LDH, and CRP values) for all the patients showed that the p values of ROC for the individual parameters as compared to the outcomes were found to be highly significant (p<0.00) as given in table 3. Table 4 shows that a statistically significant difference ($p \leq 0.05$) was found in Age, CRP, LDH, D-Dimers, and DLC in relation to patient outcome. Application of Chi-square test showed a positive association of oxygen requirements with the patient outcome with $\chi^2(4) = 174.62$, $p< 0.001$(table 5). The patients with higher oxygen requirements had a higher mortality rate as shown in Fig 1.

The sensitivity and specificity of the DLC score for the outcome of oxygen dependency came out to be 82.2 % and 80% respectively, however, for mortality as an outcome it was found to be 96% sensitive but only 32.7% specific (fig 2).

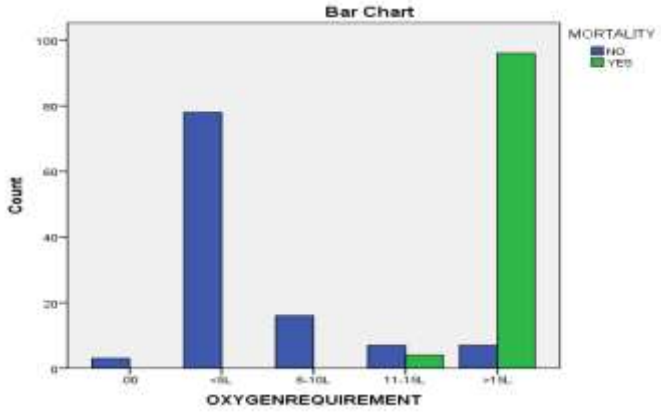

Figure 1: Oxygen requirement and mortality

Table I: Characteristics of study participants (n=213)	
Variables	Number (%)

Male Female	119(59.9) 94(44.1)
Covid PCR Status Positive Negative	 193(90.6) 20(9.4)
HRCT Findings Suggestive OF COVID HRCT not done Atypical for COVID	 45 (21.1) 167(78.4) 1 (0.5%)
Oxygen Dependence Yes No	 208(97.6) 05 (2.34)
Oxygen Requirement No requirement <5L 6-10L 11-15L >15L	213 5(2.34) 78 (36.6) 16(7.5) 11(5.2) 103(48.4)
BIPAP	49(23)
Invasive Ventilation	24(11.3)
Mortality	100(46.9)

Table II: Mean scores of demographic and laboratory parameters of the study participants (n=213)

Variables	N	Mean+ SD	Minimum	Maximum
Age in Years	213	59.9±15.71	20	95
D-DIMER (ng/ml)	213	2856.22±3675.25	50	13800
LDH (IU/L)	213	506.92± 321.44	145	2998
CRP(mg/dl)	213	101.66± 56.53	9.20	200
DLC Score	213	3468.11±3750.70	218.40	3117.50

Table III: ROC and p values in both the outcome groups (n=213)

Variable	All Patient	Outcome		Roc Cutoff	Roc Range	*p* Value
		Discharge	Death			
CRP (mg/dl)	101.66± 56.53	88.74±56.52	116.26± 53.13	0.649	0.57-0.72	0.000
LDH (IU/L)	506.92± 321.44	419.50±205.94	605.7±393.32	0.688	0.61-0.75	0.000
D-DIMER (ug/ml)	2856.22 ± 3675.25	1961.42 ± 3136.31	3867.35 ± 3981.26	0.687	0.61-0.75	0.000
DLC Score	3468.11 ±3750.7	2466.57± 3181.99	4599.84 ±4028.85	0.721	0.65-0.79	0.000

Table V: Oxygen requirement in association with patient outcome (n=213)

Variable	Responses	Frequency (Percentages) of groups		χ^2	*p* Value
		Discharged (n = 113)	Death (n = 100)		
Oxygen Requirement	No Requirement <5L 6-10L 11-15L >15L	5 (4.4) 78 (69.0) 16 (4.2) 7 (6.2) 7 (6.2)	0 (0.0) 0 (0.0) 0 (0.0) 4 (4.0) 96 (96.0)	176.59	.000**

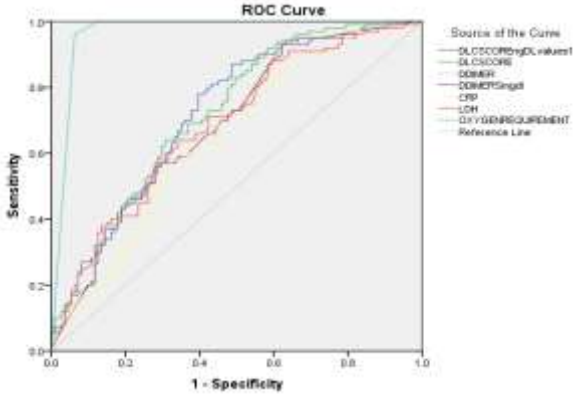

Figure 2: ROC curves for CRP, D Dimers, LDH, DLC score, and Oxygen requirement.

Discussion

COVID-19 began in December 2019, from Wuhan China and thereafter it spread globally in no time and affected the lives of millions of people worldwide.[13] It has been studied extensively over the last three years, and over these three years, several variants were identified among which delta variant was found to be the most lethal. The main pathophysiology includes a thrombo-inflammatory condition causing endovascular damage and release of several cytokines leading to a cytokine storm and thus the multisystem disease. [14]

There are several inflammatory biomarkers that have been extensively studied in COVID-19 infection and their correlation with the severity of the disease. The most commonly used being the C- Reactive

Protein (CRP), Lactate Dehydrogenase levels (LDH), D- dimers, Serum ferritin, and Interleukin 6 levels, etc.[15,16] We tried to evaluate the role of CRP, D dimers and LDH on the final outcome of the hospitalized patients. D- dimers are associated with an increased risk of thrombosis and had been previously studied in community-acquired pneumonia. It has also been reported to be associated with poor outcome and increased mortality in COVID-19.[17] LDH is an enzyme that is released into the circulation as a result of damage to cytoplasmic membrane because of either a viral infection or inflammation.[18] CRP is one of the proteins synthesized in the liver in response to inflammation and thus leads to activation of the complement pathway.[19]

In our study, we found that all these inflammatory markers i.e. D dimers, LDH and CRP had a significant correlation with the final outcome i.e. mortality in the hospitalized patients with Spearman Rho values of (0.326, 0.328, and 0.266) respectively with a p-value (0.000).

Similar findings were observed in other international and national studies as well. One study done in Peshawar CMH by Arshad et al showed that these three markers i.e., CRP, LDH, and D dimers predict mortality in hospitalized patients. This study showed that Area under Receiver Operating Characteristic Curves and 95% confidence intervals for serum C-reactive protein, and LDH was 0.909 (0.854-0.964) and 0.863 (0.785-0.942)[5], respectively. In our study the AUROC were 0.686(0.61-0.75) for D dimers, 0.688(0.61-0.75) for LDH and 0.649(0.517-0.72) for CRP respectively. The values were slightly lower in our study group compared to this study. Another study done by Mari Muthu AK et al in India in 2021 showed results that are comparable to our study with AUROC curves i.e. CRP 0.668(0.551-0.785) and D dimers 0.739(0.641-0.836) [20]

In this study, we also tried to combine the score of these parameters and formulate a new scoring system for the impact on the overall outcomes and it was found that the combined score of DLC had a stronger correlation with mortality than the individual parameters and had a better AUROC curve cut-off as compared to individual parameters i.e., ROC values of 0.718(0.65-0.78). Another study that used these combination scores was done by Kaftan AN in Iraq in 2021 which used four inflammatory markers i.e. D dimers, LDH, CRP, and ferritin. They used different combination scores for these four variables with the highest ROC values for CRP + LDH + ferritin + D dimer; AUC: 0.85 75% sensitivity and 87% specificity.[21]

Various meta-analyses have shown that high levels of CRP, ferritin, LDH, and D-dimer are associated with poor outcomes in COVID-19.[22] Similar findings showing the effect of these markers on mortality were also seen in two other studies done in Lahore by Junaid K, et al and Mahmud T et al.[23,24]

Another important fact is that COVID-19 leads to silent hypoxia. The majority of the patients that we received in the hospital were already quite hypoxic, had higher oxygen requirements, and thus had a higher mortality rate as compared to those who had low oxygen requirements. It also suggested that higher the inflammatory burden, the greater the lung damage and oxygen requirement and thus increased risk of going either on the BIPAP or the Mechanical Invasive Ventilation as seen in our study results. A meta-analysis of 26 worst affected countries by COVID done by Mansab et al in 2021 showed that the more the patient is hypoxic at presentation i.e. <90 SpO_2 the more the chances to have a poor outcome.[25]

Our study thus highlights the importance of early recognition of hypoxia and its treatment as well as the impact of the different commonly used inflammatory markers on the outcome of hospitalized patients. It has also been reported that actively treating the patients with anticoagulants, steroids, and antivirals at the earliest possible ensures better disease outcomes.

Limitations

HRCT and ferritin levels could not be done in all patients due to financial constraints. Another limitation of the study is that it was a retrospective study so the cause and effect i.e. the trend of fall in the inflammatory markers and the clinical outcome of the patient could not be assessed as well as the effect of the different treatment modalities could not be evaluated.

Conclusion

D-dimer, LDH, and CRP individually as well as a combined score of the inflammatory markers (DLC)score and Oxygen requirement had a significant correlation with the mortality of the COVID-19 patients. Judicious use of these markers may help in determining the severity of COVID pneumonia and thus the final outcome of the patient.

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