# Increased Serum Inflammatory Markers have worsened Clinical Outcomes and Mortality in Sars-Cov-2 Infection Irrespective of High or Low Serum Viral Load

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### ABSTRACT

**Aim:** To determine the viral load in the patients admitted in Covid-19 isolation and its correlation with the inflammatory markers and the following clinical outcome.

**Methodology:** A retrospective study was conducted in the Pathology Department of Doctors Hospital and Medical Centre in Lahore, Pakistan from November 2020 to January 2021. IRB approval was granted. A total of 86 patients met the inclusion criteria for the study. Data was analyzed using research tool SPSS 24.

**Results:** Increased serum viral load in SARS-CoV-2 infection showed positive correlation with inflammatory markers IL-6 (P =0.04) and D-dimer (P =0.029). Inflammatory markers LDH, Ferritin, Procalcitonin, D-Dimers and viral load itself (CT) all correlated with higher mortality while IL-6 did not.

**Conclusion:** Serum viral load in patients infected with SARS-CoV-2 correlates with higher mortality rates itself and also raises certain inflammatory markers (IL-6, D-Dimers), which are independently accountable for causing higher mortality as well. Hence, increased inflammatory markers resulted in poor prognosis regardless of high or low viral load. Their correlation with mortality can still serve as prognostic indicators.

Keywords: viral load, inflammatory markers, Covid-19, mortality

# INTRODUCTION

Coronavirus disease or COVID-19, caused by SARS-CoV-2, is an on-going challenge for healthcare workers across the world. COVID-19 has a diverse clinical spectrum as it can range from an asymptomatic state in > 80% cases to moderate or severe disease that may progress to ARDS and multisystem organ dysfunction. This can ultimately require intensive medical intervention and inevitably lead to fatal outcomes. <sup>1</sup> Although the exact pathogenesis is still unknown, many clinical, demographic, immunological and clinical factors have shown a diverse spectrum of the disease<sup>2</sup>.

SARS-Cov-2 diminishes the immune response of the body considerably leading to an impaired immune response, as well as causing a hyper-inflammatory state among the patients with severe disease. Progression to severe disease classically leads to raised levels of inflammatory markers such as ferritin, procalcitonin, IL-6 and D-dimers. Inflammatory cytokines IL-8, IP-10, monocyte chemoattractant protein 1 (MCP-1), interferon (IFN)-γ, and IL-1RA were also found to be significantly higher<sup>3</sup>. Other parameters include lymphopenia, thrombocytopenia and coagulopathies.

Coronavirus can be detected from upper and lower respiratory tract, including both the nasopharyngeal and oropharyngeal tracts. In addition, it can also be found in sputum and bronchial fluid. Bronchoscopy may be performed in some cases to obtain samples for

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Received on 13-02-2021 Accepted on 27-06-2021 microbiological cultures but it requires a highly trained staff. Upper respiratory specimens are easy to collect specially in a facility with limited resources. SARS-CoV and MERS-CoV RNA are also detected from stool, urine and blood specimens, although generally less reliable than from respiratory specimens. Nasopharyngeal specimen is the most specific and is the gold standard for the diagnosis of Covid-19<sup>4</sup>.

The viral load is expressed as cycles threshold (CTs) in real time PCR<sup>5,6</sup>. Real time PCR-CT values represent the number of amplification cycles which are required for the target gene to reach a certain threshold level. However, the CT values are affected by the viral assay itself as well the artifacts in the sample which affect the amplification of the gene<sup>7</sup>.

Previous studies have shown that when patients with a higher viral load at presentation were compared to those with a lower viral load at presentation, they were found to have a lower absolute lymphocyte count, but a higher CRP, IL-6, LDH, D-dimers and other inflammatory markers<sup>8</sup>. This is associated with increased mortality among these patients. However, there is limited data available in our country.

The purpose of this study was to determine the viral load in the patients admitted in COVID-19 isolation and its correlation with levels of inflammatory markers such as IL-6, procalcitonin, ferritin, LDH and the clinical outcome that followed. This will help in better prediction of the prognosis guide and improvement in management protocols.

### METHODOLOGY

The study was conducted retrospectively in the Pathology Department of Doctors Hospital and Medical Centre in Lahore, Pakistan. IRB approval was granted and the hospital data of 3 months was acquired from November 2020 to January 2021. All the patients who were tested positive by the PCR test method for coronavirus were included in our study. Symptomatic patients who were PCR negative and asymptomatic patients who were PCR positive were excluded. The epidemiological and clinical data of all the patients was obtained from the available records. Any patients with co-morbidities that included diabetes, hypertension, chronic kidney disease, chronic liver disease or any other metabolic disorder were also excluded. A total of 86 patients met the inclusion criteria for the study.

The nasopharyngeal swab of the patients was analyzed via real time PCR to detect the viral load. The cycles threshold (CTS) was used for the determination of viral load. The values of acute phase reactants of all these patients were noted at the time of presentation. These included IL-6, ferritin, procalcitonin, D-Dimers and LDH levels. The complete blood count parameters including the absolute lymphocyte count, platelet count and Hb levels were recorded at the time of presentation.

The clinical condition of the patient was correlated with the viral load and the inflammatory markers at the time of presentation. The clinical outcome that occurred during the time in isolation ward was recorded and the viral load of each patient was also correlated in a similar manner.

The data obtained was analyzed using SPSS version 24. The continuous variables were subjected to descriptive analysis to obtain percentages, mean and standard deviation. Categorical variables were analyzed in terms of frequencies and correlation between the variables was obtained using chi-square test where p value of less than 0.05 was considered significant.

#### RESULTS

A total 86 patients with Covid-19 were included in the study, following the inclusion criteria of which 65 were male and 21 were female patients with a mean age of 63 years old. Viral load among all the patients was analyzed as a continuous variable as well as a categorical variable to determine the relationship with acute phase reactants and the mortality rate. The mean value of viral load was 26.65  $\pm$ SD 4.47 with a minimum and maximum value of 14.60 and 34.30 CT respectively. The relationship of viral load with acute phase reactants shows a positive correlation with IL-6 and D-dimers (Table 1).

The mortality rate was calculated to be 14.2% with a total of 25 patients dying during their illness and the remaining 61(70.9%) patients recovered. The relationship of mortality and acute phase reactants was calculated (Table 2). A positive correlation was seen with raised values of LDH, procalcitonin, D-dimers and ferritin. Whereas, IL-6 values seemed to be insignificant. The cycle threshold value of even less than 30 has shown positive correlation with mortality rate.

Complete Blood Count parameters and Acute Phase Reactants values		Viral Load				P-value
		Less	Less than 30 CT		More than 30 CT	
		N	N %	Ν	N %	
Hemoglobin (g/dL)	Less than 10	28	84.8%	5	15.2%	0.055
	More than 10	35	66.0%	18	34.0%	
Absolute lymphocyte count 10^3UI	More than 0.8	26	57.8%	19	42.2%	0.001
	Less than 0.8	37	90.2%	4	9.8%	
Platelet count (10 <sup>°</sup> uL)	150 to 400	33	66.0%	17	34.0%	0.73
	Less than 150	30	83.3%	6	16.7%	
LDH (u/l)	122 to 222	29	72.5%	11	27.5%	0.883
	More than 220	34	73.9%	12	26.1%	
Procalcitonin (ng/ml)	Less than 0.046	38	66.7%	19	33.3%	0.146
	More than 0.046	24	85.7%	4	14.3%	
IL6 (pg/ml)	Less than 7	19	90.5%	2	9.5%	0.040
	More than 7	44	67.7%	21	32.3%	
D-Dimers(ng/ml)	Less than 0.5	27	87.1%	4	12.9%	0.029
	More than 0.5	36	65.5%	19	34.5%	
Ferritin (ug/ml)	Less than 335	25	67.6%	12	32.4%	0.300
	More than 335	38	77.6%	11	22.4%	

#### Table 1: Relationship of Viral Load

Table 2: Relationship of mortality

Complete Blood Count parameters and Acute			Mortality				
Phase Reactants values		Red	Recovered		Died		
		N	N %	N	N %		
Hemoglobin (g/dL)	less than 10	20	60.6%	13	39.4%	0.096	
	more than 10	41	77.4%	12	22.6%		
Absolute lymphocyte	More than 0.8	42	93.3%	3	6.7%	<0.001	
count 10^3uL	Less than 0.8	19	46.3%	22	53.7%		
Platelet count	150 to 400	45	90.0%	5	10.0%	< 0.001	
(10° uL)	Less than 150	16	44.4%	20	55.6%		
LDH (u/l)	122 to 222	38	95.0%	2	5.0%	< 0.001	
	More than 220	23	50.0%	23	50.0%		

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Procalcitonin (ng/ml)	Less than 0.046	47	82.5%	10	17.5%	0.002
	More than 0.046	14	46.4%	15	53.6%	
IL6 (pg/ml)	Less than 7	18	85.7%	3	14.3%	0.086
	More than 7	43	66.2%	22	33.8%	
D-Dimers(ng/ml)	Less than 0.5	28	90.3%	3	9.7%	0.003
	More than 0.5	33	60.0%	22	40.0%	
Ferritin (ug/ml)	Less than 335	35	94.6%	2	5.4%	< 0.001
	More than 335	26	53.1%	23	46.9%	
Viral Load (CT)	Less than 30 CT	41	65.1%	22	34.9%	
	More than 30 CT	20	87%	3	13%	0.048

# DISCUSSION

The ongoing pandemic of Covid-19 has a spectrum of disease severity. It can present as asymptomatic disease, severe pneumonia and even death. Hence, it is important to monitor the severity of the disease along with prognostic factors for effective management.

Recently several studies have been done to determine the relationship of inflammatory markers with the severity of the disease in terms of viral load. <sup>9</sup> In our study we discussed the correlation between the viral load and the adverse clinical outcome of coronavirus infections. The viral load was very important in determining the dynamics of the disease in the body, which led to better surveillance of the patients. Previously, positive correlation of viral load with disease severity had been done in other diseases such as HIV and HINI influenza virus (commonly known as swine flu)<sup>10</sup>.

The data from our study showed that hematological parameters including lymphocytopenia and neutrophilia were directly related with viral load detected through nasopharyngeal swab. High mortality rate was seen in patients with low platelet count and lymphocytopenia. Various studies have targeted the correlation of hematological parameters with disease severity. A study done in the Chinese city of Wuhan has shown direct association of low platelet count with severe covid infection. Also, that study proved the role of hematological parameters in defining the severity and prognosis of the disease.<sup>11</sup> Similarly, a recent study conducted in Lahore, Pakistan shows that lymphocytopenia, thrombocytopenia and neutrophilia are associated with poor clinical outcome of the disease<sup>12</sup>.

The inflammatory markers have a crucial role in early stages and progression of viral infections. We assessed various inflammatory markers such as IL-6, D-Dimer, LDH, procalcitonin and ferritin levels in our patients. The levels of IL-6 and D- Dimers were found to be raised in patients with higher viral load (CT less than 30) and it shows that Covid-19 provokes an immune response leading to cytokine release.

LDH is an inflammatory marker associated with increased cellular damage. Various studies have established the relationship of high LDH levels and severe covid infections<sup>13</sup>. In our study, the patients who had high levels of LDH at admission showed high mortality rate. However, we did not see any correlation of LDH levels with viral load. A previous study has shown that patients who had high levels of LDH at admission were more likely to develop ARDS and therefore, is related to severe infection<sup>14</sup>.

Procalcitonin is another inflammatory marker released in response to high levels of IL-6<sup>15</sup>. Recently, its role in coronavirus infection has been established to monitor disease severity. Several studies have shown that increased levels of procalcitonin indicate that the patient is suffering from severe disease. <sup>16</sup> Also, the progressively raised levels of procalcitonin during the course of illness are considered as a better predictor of disease progression with a high predictive value. These results are in agreement to our study in terms of disease severity and high mortality observed in patients with increased levels of procalcitonin.

IL-6 is an important cytokine in controlling immune response of the body possessing proinflammatory and antiinflammatory properties. In our study, patients who had high viral load had high levels of IL-6 and severe respiratory symptoms. <sup>17</sup> Previous studies on SARS patients have shown that high level of proinflammatory markers including IL-6 is related with increased damage to the lungs<sup>18</sup>. Furthermore, recent studies on IL-6 in relation to Covid-19 infection in Wuhan have shown strong correlation between the severity of the infection and IL-6 levels<sup>19</sup>.

The increased levels of D-Dimers can be used as a marker of thrombotic events as well as several others infections. However, its diagnostic role as a thromboembolic marker in patients of Covid-19 has not been proven yet. A recent study has suggested that anticoagulation therapy is necessary for the patients with raised D-Dimers levels as the risk of pulmonary embolism becomes high in such patients<sup>20</sup>. Besides the thromboembolic risk, D-Dimers can be used as independent predictors of mortality in severe COVID-19 patients. A study in Wuhan has proved the role of D-Dimers as a mortality predictor, distinguishing the moderate and severe COVID-19 infection, as well as providing guidance for anticoagulation therapy<sup>21</sup>.

The role of ferritin and iron metabolism in COVID-19 infection is still controversial. It has been seen in various studies that patients with severe COVID-19 infections have high levels of ferritin in contrast with other infections but it is not considered as a standard predictive inflammatory marker in Covid-19 infection yet<sup>22</sup>. In addition, COVID-19 is a multisystem disease targeting different organs of the body via the ACE receptors. The role of inflammatory markers in predicting disease severity and mortality has been established in several studies and can be helpful for better patient outcome<sup>23</sup>.

Strong association of nasopharyngeal viral load was found with the mortality rate in our patients. This was consistent with one of the pioneer studies which showed hospital mortality rate as 34% in group 1 compared with 13% in group 2 with decreased viral load<sup>24</sup>. Similar results were seen in another study that showed a 35% mortality rate in patients having CT value less than  $25^{25}$ . A study conducted on older adults with Covid-19 infections also found CT value to be an independent predictor of mortality<sup>26</sup>.

**Limitations:** The limitations of our study include a small sample size and detection of only nasopharyngeal viral load. In addition, the serial levels of the inflammatory markers were not taken which could reflect better prediction of disease outcome.

#### CONCLUSION

Hematological parameters and inflammatory markers can serve as novel prognostic factors and should be included in routine investigations of infected patients. A higher viral load can cause significant elevations in some inflammatory markers such as D-Dimers and IL-6. A rise in viral load also correlated with increased mortality. Serum inflammatory markers such as D-Dimers, LDH, ferritin, procalcitonin can serve as biomarkers for severity of disease and progression. Raised D-dimers in particular correlate with increased mortality and their levels guide physicians on use of anticoagulation. In addition, this study also found correlation of hematological parameters with disease severity and mortality, guiding physicians to obtain regular hematological testing. Furthermore, patients with high viral loads as well as raised inflammatory markers did worse clinically with high morbidity and mortality. However, increased inflammatory markers resulted in poor prognosis regardless of high or low viral load. Hence, viral load and raised inflammatory markers with their correlation with mortality can serve as prognostic indicators for physicians.

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