Prognostic Factors and Association of Inflammatory Biomarkers with Severity and Mortality in COVID-19

Hamzullah Khan, Shahtaj Khan, Huma Riaz, Asad Rehman Khattak

ABSTRACT:

Objectives: To determine prognostic values and association of inflammatory markers with severity and mortality in COVID-19 in a hospital based study.

Study design and setting: This retrospective study was conducted from 1st June 2020 to 30th Sept 2020 in Department of Pathology, Qazi Hussain Ahmed Medical Complex, Nowshera and Medical Teaching Institute, Hayatabad Medical complex Peshawar.

Methodology: Out of 215, 71 cases were selected that had all relevant information's on chart available in Blood bank and department of Pathology.

Results: Out of 71 patients, 54 (76.1%) were males and 17 (23.9%) females. Thirty five (49.3%) had age>55 years while 31 (43.7%) were in age range 36-55 years. The frequency of the different blood groups were; 25 (35.2%) B+ blood group followed by 19 (26.8%) A+ and 14 (19.7%) O+ blood group. The AUC for d-dimers was (0.725, 95% CI 0.599-0.855) followed by CRP (0.565 95%CI 0.422-0.7.8) and ferritin (0.519 95%CI 0.36-0.679). The median values of d-dimers was significantly higher in the deceased as compared to the survivors (p<0.05- Mann Whitney U test).The CRP and ferritin levels were not significantly different in study groups. There was a significant positive uphill correlation of the hospital stay with higher values of d-dimers (p=0.01, r_s= 0.287).

Conclusion: D-dimer is a main prognostic factor that predicts mortality in COVID-19 followed by CRP and serum ferritin levels. Male gender and patient with age>60 are at risk of worst outcome under the impact of deranged values of inflammatory mediators. Hospital stay and blood group of patients have no relation with outcome.

Keywords: CRP, Mortality, COVID-19, Ferritin, D-dimers, Inflammatory markers.

INTRODUCTION:

Covid-19 is a respiratory disease that was reported to the World Health Organization in Dec 2019 from the metropolitan city of Wuhan, China. The World Health Organization (WHO) declared global emergency due to rapid rise in cases of COVID-19 in China and nearby countries by the mid of February 2020. By the date 20th Feb 2020 the number of laboratory confirmed cases reached to 60,000 in China with 1700 deaths.[1]

Globally, Since the 31st December 2019 and till date (22nd January2021), a total of 98,353,744 cases have been reported worldwide, in accordance with the definition of the Centre of Disease Control (CDC) of COVID-19 cases, with 2,106,818 deaths.[2]

In Pakistan, the literature so far covering the prevalence and incidence is deficient. By 22nd Jan 2021, reported data from government sources declares 529,000 confirmed cases with 11204 deaths. Khyber Pukhtunkhwa has reported 64651 cases and 1823 deaths.[3]

Increasing scientific research in field of COVID-19, the abnormalities in routine laboratory investigations, particularly the baseline investigations, has the potential to indicate the severity of the disease in a quick, easy, affordable, accessible and economical way.[4] Consequently the inflammatory markers like serum ferritin, d-dimers and C-reactive proteins, prolactin and erythrocytes sedimentation rate have been reported to predict the disease severity and mortality in

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COVID-19.[5] The number of patients are increasing in the second wave and critically ill patients needs ICU treatment that causing a major challenge. Therefore timely identification of severe disease is required with identification of values of prognostic markers to label patients with high risk for mortality.

Authors have reported from the city of Wuhan, China, the hyperferritnemia as predictor of mortality and severity of the disease.[6] The literature supported the fact that on admission to isolation wards the average ferritin concentration in COVID-19 patients was >800 ìg/L, that was 5.3 times higher in patients with severe disease at time of presentation. Similarly in-hospital mortality was higher in patients with serum ferritin levels >1400 ìg/L as compared to the survivors with <1400 ìg/L ferritin.[7,8]

A local study conducted in Peshawar, Pakistan concluded that more than 80% of the COVID-19 patients admitted in isolation and ICU had abnormally higher readings of serum ferritin. There was a statistically significant correlation of deranged ferritin levels with an increase in age of patient and with worst outcome in terms of death. Female gender more protective with higher chances of survival with deranged serum ferritin levels in all age groups compared to male gender.[9]

Potentially the linkage of COVID-19 and venous thromboembolism is well described and reported by number of authors with significant alteration of the hematological parameters in corona infection. Some have described the positive association of mortality and morbidity with an increase in the level of d-dimers in COVID-19.[10] A study reported the AUC for plasma CRP levels (0.896) on ROC curve which was significantly higher than age (0.833), ANC (0.820) and platelet count (0.677) in outcome prediction with cutoff values of 41.4 (Sensitivity 90%, specificty 77%).[11]

The rapid spread of the pandemic necessitates to prioritize patients in risk groups based on levels of these markers to detect the severity of the disease well in time to reduce mortality.

Facilities of costly cytokine analysis is not available everywhere in most of the healthcare facilities. Hence markers like CRP. D-dimers, ferritin are of great interst of the researcher for prognostic values to predict the severity of the pandemic.

Therefore present study was conducted to determine the prognostic values and association of inflammatory markers with severity and mortality in COVID-19 in a hospital based study

METHODOLOGY:

This retrospective study was conducted from 1st June 2020 to 30th Sept 2020 in Department of Pathology, Medical Teaching Institute (MTI) Qazi Hussain Ahmed Medical Complex, Nowshera and Postgraduate Medical Institute,

MTI, Hayatabad Medical complex Peshawar. Out of 215 files, 71 COVID-19 patients had complete information and they were referred to pathology with the request of transfusion of packed cells, blood product, FFP or COVID convalescent plasma therapy' were further studied.

Patients hospitalized with COVID-19 in ICU-COVID-19, HMC were included. Patients where all inflammatory mediators were advised like d-dimers, ferritin, and CRP were advised by the treating consultant were included only, irrespective of age and gender. Similarly those patients were further observed for disease outcome in terms of satisfactory discharge or case fatality due to COVID-19 to be included.

COVID-19 patients where deficient inflammatory mediator readings were available on chart of patients, or patients expired before to be fully investigated were excluded. Similarly all patients with any type of symptoms came to emergency or outdoor patients department or even confirmed patients of COVID-19 in isolation department where readings of the inflammatory mediators were not advised were also excluded. The Serum Ferritin, d-dimer levels was measured by electro-chemiluminescence immunoassay using Roche Cobas E411 Chemistry Analyzer, using commercial kits of Roche diagnostics as per the instructions of the manufacturer.

This study was conducted in line with the research regulations, followed the sound medical practice, redetected human rights and also within the principles of declaration of Helsinki of World Medical Association.

Ethical endorsement was obtained from the institutional ethical review board of Post-Graduate Medical Institute, Hayatabad Medical Complex under notification No (316/HEC/B & PSC/2020 Dated 15th May 2020) and notification No (346/HEC/B & PSC/2020 Dated 10th Dec 2020).

For COVID confirmation, PCR results of the naso-pharyngeal swab duly reported in Public health research laboratory of the Hospital and also of the Khyber medical University (a designated Lab for RT-PCR of 2019nCoV by the Government of Khyber Pukhtunkhwa) were considered only.

Data was entered in SPSS, version 25 and descriptive and correlation statistics were applied. Numerical variables like age of patients and serum ferritin, d-dimer, CRP, age and hospital stay were presented with Mean and SD in case of normal distribution and with median and range where the data was not normally distributed.

Normality of data was assessed using Shapiro-wilk Test.

Independent t-test/Mann Whitney U test were used to determine the difference of the numerical variables (serum ferritin, d-dimer, CRP, age and hospital stay) in groups (discharged satisfactory vs expired). Chi-square test was used to show an association of blood group age and gender with disease outcome in COVID-19 patients.

Receiver operating characteristics (ROC) curve was used

to determine the relationship of clinical sensitivity and specificity of different inflammatory mediators to predict the worst outcome in COVID-19. Correlation tests using Spearman ranked correlation was used to determine the correlation of quantitative variables (serum ferritin d-dimer, CRP, age and hospital stay).

RESULTS:

Out of 71 patients who were selected for plasma therapy, 54 (76.1%) were males and 17 (23.9%) were females. Thirty five (49.3%) had age>55 years while 31 (43.7%) were in age range 36-55 years and 5 (7%) had age less than 35 years. Majority 25 (35.2%) had B+ blood group followed by 19 (26.8%) A+, 14 (19.7%) O+ blood groups. Sixty one (85.91% had Rh+ while 10 (14.08%) had Rh- blood. Out of total, 25(35%) expired during hospitalization.

The data distribution was assessed with Shapirowilk test and we found that distribution of age in both genders was normally distributed (p-value>0.05) while the distribution of all the three inflammatory markers was in a skewed pattern (p-value<0.05).

While assessing the association of different demographic and hematological factors with outcome, we observed that there was no significant association of gender (p-0.993), age (0.892) and blood group (0.855).

The difference in Mean/median values of different inflammatory markers and hospital stay with disease outcome,

it was observed that the median value of d-dimers was significantly higher in the deceased as compared to the survivors (p<0.05- Mann Whitney U test). No significant difference in median value of ferritin was observed between survivors versus non-survivors (Mann Whitney U test, p>0.05). The Mean with SD of CRP and hospital stay was not significantly different in groups (survivors vs expired) with (independent t test, p=0.566, p=0.423 respectively). (Table 1)

The cut off values of inflammatory mediators for predicting mortality is shown in Table 2. The table further shows the AUC for d-dimers was (0.725, 95% CI 0.599-0.855) followed by CRP (0.565 95%CI 0.422-0.7.8) and ferritin (0.519 95%CI 0.36-0.679).

Correlation matrix showing the correlation coefficient for different quantitative variables in table 6. There was a positive uphill significant correlation of hospital stay with higher values of d-dimers (p=0.01, r_s= 0.287). Unlikely d-dimer by assessing the correlation of the hospital stay with CRP and ferritin was not statistically significant. (Table 3)

The ROC curve showing the area under curve for different inflammatory markers in figure 1.

DISCUSSION:

Male predominance (76.1%) was found in this study. Likewise 35 (49.3%) of the patients presented with age >55 years and 25 (35.2%) had B+ blood group followed as major

Table 1. Difference in inflammatory markers in COVID-19 in study groups

| Hematological Markers	Improved or (survivors) (N=42)	Died or (non-survivors) (N=25)	p-value	Test of significance
C-Reactive Protien (CRP) Mean ± SD	15.18±12.10	16.86±10	0.566	Independent T Test
Serum Ferritin Median	1518.4	1363.12	p>0.05	Mann Whitney U Test
d-dimers **Median**	28.89	135.4	p<0.05	Mann Whitney U Test
Hospital Stay Mean ± SD	9.6±8	7.92±8	0.423	Independent T Test

Table 2. ROC statistics for predicting mortality in COVID-19 patients

Test Result Variable(s)	Cutoff values	Sensitivity (%)	Specificity (%)	Area (AUC)	Std. Error	Asymptotic 95% Confidence Interval		Sig. (p-value)
						Lower Bound	Upper Bound	
D_Dimer	2.25	82	80	0.725	0.064	0.599	0.85	0.002
CRP	8.7	72	57	0.565	0.073	0.422	0.708	0.378
Ferritin	1103	62	60	0.519	0.081	0.36	0.679	0.795
The test result variable(s): D_Dimer, CRP, Ferritin has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.								
a. Under the nonparametric assumption								
b. Null hypothesis: true area = 0.5								

Table 3. Correlation matrix showing the the Spearman's correlation coefficient (rs) for Hospital stay with different inflammatory markers

		CRP	Ferritin	D_Dimer
Ferritin	**r_s**	0.196		
	p	0.111		
D_Dimers	**r_s**	0.103	0.189	
	p	0.405	0.123	
Hospital stay (days)	**r_s**	0.037	0.044	.287*
	p	0.764	0.719	0.017
Number of patients		67	68	68

Figure 1. ROC Curve for inflammatory markers
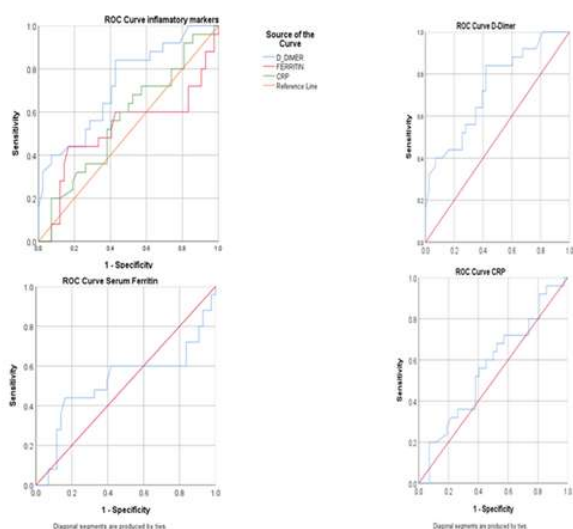
blood groups. The target population was bit biased as included patients who were selected for plasma therapy and by default in initial phases only serious patients were selected for plasma therapy and was thought to be one of the most safe therapy for the serious patients. Total n=25 cases out of 71 of the target population whose files were present in the department reported expired during hospitalization. This mortality reflects the higher rate and as mainly of those patients had serious disease. Reportedly 215 cases received transfusion of blood products for different complication and needs. It was observed that the AUC for d-dimers was (0.725) followed by CRP (0.565) and ferritin (0.519) which shows the higher clinical sensitivity of d-dimer on ROC curve to predict mortality in COVID-19. Similarly the median value of d-dimers was significantly higher in the deceased as compared to the survivors (28 vs 135) with p-value<0.05. The surviving ability drops to less than 20% in age >70 with female gender confer more immunity/chances of survival as compared to male gender.

The measurements of inflammatory markers assist the physicians to predict the severity of the disease.[12] Accumulative assessment have concluded that inflammatory response play a critical role in the COVID-19 progression.[12,13]

Several inflammatory markers such as Serum ferritin, d-dimers, c-reactive proteins, Interleukin-6 have been reported with high risk of the severe disease.[13]

Gao Y et al[14] have reported that the area under curved for deranged d-dimers as 0.840, which is higher than the findings and further confers the higher clinical impact of deranged d-dimers on the worst outcome in COVID-19. Similarly a meta-analysis reported d-dimer 3.9 times higher in the exposed groups who meet worst outcome in term of death.[15] The abnormally deranged serum ferritin levels may be crucial for in COVID-19 patients. A study from Wuhan reported patients who had d-dimer>2 µg/mL had higher fatality than patient who presented with d-dimer>2 µg/mL (p<0.05).[16]

A study appeared in JAMA recommended therefore that it would be more beneficial to use the cut off values of the d-dimers for specific population and then for age and gender in the scenario of the COVID-19. That must be added then to the national surveillance system to be used for population studies to determine the high risk group more vulnerable to meet worst outcome in COVID-19.[17]

A local study reported the probability of worse outcome in term of death was 2.06 times more in patients with dimers > 0.5 µg/ml (OR = 2.06) with a relative risk of 1.1.[18]

The serum ferritin levels are reported high and non stoppable in hospitalized patient with COVID-19. A study reported an increase in the ferritin levels exceeding the upper limit of detection, with increase in hospital stay. Similarly ferritin levels were significantly higher (p<0.05) in critically ill patients (ICU patients) as compared to severe diseased group (Non-ICU).[19] However in present study it was observed a higher ferritin in both groups survivors vs. deceased and was not statistically different in both the groups. The reason may be that hospital is the end stage referral hospitals for COVID-19 care and usually received referral at very end stage with severe disease. A study from China reported higher levels of the serum ferritin in patients died due to COVID-19 infection as compared to the patients discharged. But a trend of high ferritin was noted in COVID-19 patients throughout the clinical course as observed in both the groups (Discharges vs Deceased).[20] Another local study that was reported from the Peshawar Pakistan showed that 80% of the COVID-19 patients had deranged levels of serum ferritin at presentation to hospital. These abnormally higher ferritin levels had a statistically significant relationship with an increase in age of patients and outcome in terms of death.[9]

Furthermore it was the only hospital that was allowed and authorized for plasma exchange and included patients from ICU. Therefore at entry to the ICU, majority of the patients were in cytokine storm with high inflammatory markers. Hence, ferritin values was higher in both groups irrespective of outcome and the impact on outcome was less specific than d-dimer. High d-dimer values can be observed in severe

disease after long time while the values of the ferritin and d-dimer decreased with an increase in the disease duration. Likewise a higher in-hospital mortality has been reported in patient with high levels of CRP (>101mg/dl).[20] Their findings were higher than the results of this study where the mean +SD of CRP was 16.86+10mg/dl, but the difference was not statistically significant.

A retrospective study from Karachi reported C-reactive protein (CRP) levels predicting ICU admission with area under the curve (AUC): 0.806, positive predictive value (PPV): 85.1%. [21]

Thus findings of this study support the literature reported from the metropolitan cities affected by COVID-19. The initial readings of the inflammatory markers are predictive of severity and mortality for this pandemic, however the preference in values of d-dimer with ferritin and CRP is different in different studies reported from different quarter/regions as evident from literature/research studies.[16, 19, 21,22] These markers values need to be specified for different age and gender groups of the respective populations in order to know the severity of the disease. There were some limitations of the study. Smaller sample size restricts us to predict the application of this study on larger population, including more inflammatory markers like LDH etc. Studies executed with larger sample size can estimate the true population impact. Therefore it is suggested that future studies should cover large population with representation to predict/suggest the findings for decision makers.

CONCLUSION:

D-dimer values can be relied as best prognostic indicator at all stages of the disease. D-dimer is a main prognostic factor that predicts mortality in COVID-19 followed by CRP and serum ferritin levels. Male gender and patient with age>60 are at risk of worst outcome under the impact of deranged values of inflammatory mediators. Hospital stay itself has no significant correlation with outcome. Blood group itself does not predict about the severity or fatality of the disease.

Hamzullah Khan: Conception, design, analysis and/or interpretation of data writing the manuscript
Shahtaj Khan: Design, Data analysis and critical review
Huma Riaz: Data Collection analysis and/or interpretation of data
Asad Rehman Khattak: Data Collection

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