ORIGINAL ARTICLE
DO LABORATORY BIOMARKERS PREDICT SURVIVAL IN SEVERE COVID-19? A CROSS-SECTIONAL STUDY

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Background: This study aims to compare lymphocyte count, C-reactive protein (CRP), ferritin, Lactate Dehydrogenase (LDH) and D-dimer among survivors and non-survivors of severe COVID-19. **Methods:** This retrospective cross-sectional analytical study included 69 patients for whom a record of the biomarkers and survival status was available. Baseline and peak values were selected for serum CRP, ferritin, LDH and D-Dimer. Baseline and trough lymphocyte counts were selected. Data were analyzed using SPSS version 21. Mean and standard deviation were used to compare the biomarkers with paired t-test. p-value <0.05 was taken as significant. **Results:** The mean age of the study population was 55.5±9.1 years and 50 (72.5%) were male. Among survivors, the increase in CRP level was not significant (from 15.80±9.8 mg/dl to 17.87±8.4 mg/dl, p=0.45) while it was significant in non-survivors (from 16.68±10.90 mg/dl to 20.77±12.69 mg/dl, p=0.04). There was no significant rise in LDH levels in survivors (from 829.59±499 U/L to 1018.6±468 U/L, p=0.20) while it increased significantly in non-survivors (from 816.2±443.08 U/L to 1056.61±480.54 U/L, p=0.003). The decrease in lymphocyte count and increase in D-Dimers in both the groups was significant (p=0.001). There was no significant elevation in ferritin in both the groups (p>0.05). **Conclusion:** In severe COVID-19 patients, serum CRP and LDH can be used for risk stratification and predicting survival. Lymphopenia, increase in serum ferritin and D-dimers may not predict survival.

Keywords: COVID-19; SARS-CoV-2; Lymphopenia; C-reactive protein; Lactate Dehydrogenase; D-dimer

INTRODUCTION

Corona Virus Disease 2019 (COVID-19) originated as unusual pneumonia in the visitors of a seafood market which was also selling live wild animals in Wuhan, China. Soon it was realized that human to human transmission does occur as similar symptoms appeared in those who got in contact with the cases & never visited the market.[1] Shortly after China, it spread across the globe. It was declared as a pandemic on March 11, 2020.[2] As of December 22, 2020 there are 76,023,488 confirmed cases globally and 1,694,128 deaths worldwide.[3] In Pakistan, there are 460,672 confirmed cases of COVID-19, and death tally of 9,474.[4]

Corona Virus Disease 2019 has posed medical, social and scientific challenges all over the world due to its complex and unpredictable clinical course. Multiple factors have been proposed for the complex and ill-defined course of the illness.[5] In the most of the cases, the disease is fairly mild, while in the rest it results in severe disease leading to multiorgan failure and death.[6] Apart from gender, age, blood groups, and co-morbidities, a plethora of laboratory markers have been investigated to predict the course and outcome of the disease.[7–10]

Infection with SARS CoV-2 leads to the release of cytokines and chemokines from monocytes, lymphocytes and macrophages resulting in an inflammatory response.[11] The common laboratory parameters which are supposed to be linked with worse outcome in COVID-19 include CRP, interleukin-6 (IL-6), D-dimer, fibrinogen, LDH, Cardiac Troponin, lymphocyte count, serum ferritin, serum amyloid A (SAA) and erythrocyte sedimentation rate (ESR).[12] However, there is no clarity as the results of most of the studies are inconsistent.[1,5,10,12,13] Laboratory predictors for outcome in terms of survival need a comprehensive investigation.

The course of SARS-CoV-2 in Pakistan is different from the developed world. The cases have declined (from 6,825 new cases per day on June 13th, 2020 to 450 new cases on August 24th, 2020)[4] in the last few weeks. The seropositivity is reported as 11%[14] in the general population which indicates that herd immunity is still to be achieved. On the other

hand, adherence to standards operating procedures (SOPs) for SARS- CoV-2 were also not visible in most of the areas. This discrepancy from the rest of the world could be linked to the genetics of the population as well as that of the virus. Given the different course that COVID-19 has taken in Pakistan, it is likely that the pattern of immune and inflammatory response to SARS-CoV-2 infection in our population is distinct. Keeping this in view, this study aims to analyze the pattern of different laboratory parameters between survivors and non-survivors of severe COVID-19 and compare the results with published literature.

MATERIAL AND METHODS

This study was approved by Institutional Review Board of Khyber Girls Medical College / Hayatabad Medical Complex, Peshawar, Pakistan. Electronic record of the patients was used for this cross-sectional study. All confirmed cases of severe COVID-19 as per WHO criteria[15] in COVID – intensive care unit (COVID-ICU) of Hayatabad Medical Complex (HMC), Peshawar were eligible for inclusion in the study. The data were retrieved from Hospital Information System (HIS). The laboratory markers included in the study were lymphocyte count, CRP level, serum ferritin level, serum LDH level and serum D-dimer level. The outcome of patients was classified as survivor and non-survivor. Survivor was defined as a patient who was discharged from the hospital following improvement in symptoms. Non-survivor was defined as a patient who died during hospital stay with COVID-19 as a predominant contributory factor in death. Sixty-nine patients admitted in COVID-ICU, for whom data of proposed laboratory markers and outcome was available, were included in the study. All patients received standard treatment for severe COVID-19. For every patient, baseline and peak values were selected for CRP level, serum ferritin level, serum LDH level and serum D-Dimer level. Similarly, baseline and trough levels were selected for lymphocytes. Analysis was carried out in SPSS version 21.0. Qualitative data like gender, comorbidities, and age groups were expressed as frequency and percentages while quantitative variables like age and laboratory biomarkers' levels were calculated as mean±S.D. Paired t-test was applied for comparison of means of study variables (lymphocyte count, serum CRP level, ferritin, LDH and d-dimer levels) in each group (survivors and non-survivors). p value below 0.05 was considered significant.

RESULTS

A total of 69 patients' data from COVID-ICU were studied, having a mean age of 55.5±9.1 years. Among them, 50 (72.5%) were male, 47 (68.1%) were above 50 years age and 7 (10.1%) were diabetics. (Table-1).

The lymphocyte count decreased significantly in both survivors (from 0.94±0.36x10^9/L to 0.51±0.28 x10^9/L, p=0.001) and non-survivors (from 1.24±0.80 x10^9/L to 0.61±0.55 x10^9/L, p=0.001). Among survivors, the increase in CRP level was not significant (from 15.80±9.8 mg/dl to 17.87±8.4 mg/dl, p=0.45) while among the non-survivors, the increase in CRP level was significant (from 16.68± 10.90 mg/dl to 20.77±12.69 mg/dl, p=0.04). There was no significant rise in serum ferritin among the survivors and non-survivors (p>0.05). There was no significant rise in serum LDH levels in survivors (from 829.59±499 U/L to 1018.6±468 U/L, p=0.20) while there was a statistically significant increase in serum LDH level in non-survivors (from 816.2±443.08 U/L to 1056.61±480.54 U/L, p=0.003). The D-Dimer level increased significantly in both survivors and non-survivors (from 7.2±9.8 µg/ml to 28.8±35.4 µg/ml, p=0.01 and from 8.75±14.8 µg/ml to 29.52±37.96 µg/ml, p=0.001, respectively). (Table-2)

Table-1: Demographic parameters of the study population (n=69)

Parameters	Frequency	Percentage
Gender		
Male	50	72.5%
Female	19	27.5%
Age groups		
Up to 50 years	22	31.9%
Above 50 years	47	68.1%
Comorbidities		
Diabetes	07	10.1%
Hypertension	04	5.8%
Ischemic heart disease	03	4.3%
Chronic kidney disease	02	2.9%

Table-2: Laboratory parameters in survivors and non-survivors

Parameters	Survivors (n=22)		p-Value	Non-survivors (n=47)		p-Value
	Baseline	Peak/Trough		Baseline	Peak/Trough	
	Mean ± SD	Mean ± SD		Mean ± SD	Mean ± SD	
Lymphocytes (10^9/L)	0.94±0.36	0.51±0.28	0.001	1.24±0.80	0.61±0.55	0.001
CRP (mg/dl)	15.80±9.8	17.87 ±8.4	0.45	16.68± 10.90	20.77±12.69	0.04
Ferritin (ng/ml)	1321.1±1443	2141.18±3521	0.31	1227.01±1064.1	1662.7±21	0.12
LDH (U/L)	829.5±499	1018.6±468	0.20	816.2±443.08	1056.6±480.5	0.003
D-Dimer (µg/ml)	7.2±9.8	28.8±35.4	0.01	8.7±14.8	29.5±37.96	0.001

CRP:C-reactive protein; LDH: Lactate Dehydrogenase; SD: Standard deviation

DISCUSSION

The results of the present study were based on laboratory parameters of 69 confirmed cases of COVID-19 admitted to ICU. Lymphocyte count was significantly decreased in both survivors and non-survivors ($p<0.001$). Similar findings were reported by Huang and Pranata[16] who observed that lymphopenia is found to be the key predictor for the severity of COVID-19 and is associated with poor outcomes. They reported that cases with worse outcome have a lower lymphocyte count with a mean difference = -361.06 per µL (95% CI -439.18, -282.95; $p<0.001$;) when compared to cases with favourable outcome. Research conducted by Zhou et al.[1] revealed that cases with COVID-19 who did not survive had lower lymphocyte count in comparison to survivors (0.6 vs $1.1x10^9$/L, $p<0.0001$). In contrast to Zhou *et al.* where patients with all stages of COVID-19 were studied, the patients included in this study had only severe disease. Moreover, we determined the maximum drop in lymphocyte count from baseline in both groups. The potential mechanisms of lymphopenia in COVID-19 are; direct destruction of lymphocytes by the virus, injury to the lymphatic organs,[16] abnormalities of cytokines leading to lymphocyte depletion,[17] and lactic acidosis secondary to hypoxemia.[18]

In the present study significant rise in CRP was found to be a marker of non-survival. There was significant increase (from 16.68±10.90 mg/dl to 20.77±12.69 mg/dl, $p=0.04$) in non-survivor as compared to survivors ($p=0.45$). The association of CRP with poor outcomes in COVID-19 has already been reported by Li *et al.*, Feng *et al.,* Chen *et al* and Wang.[7,8,19,20] Research suggests that CRP is one of the most important biomarkers to predict the prognosis of COVID-19.[10]

The serum ferritin increased from the baseline in survivors and non-survivors, but the rise in both groups was not significant. ($p=0.31$ and $p=0.12$, respectively). This contrasts with Zhou *et al.*[1] who have reported significantly elevated serum ferritin in non-survivors as compared to survivors (1435 ng/ml and 503 ng/ml, respectively; $p<0.0001$). Similarly, Chen *et al.*[12] have observed higher serum ferritin in patients with severe illness as compared to those with moderate disease (serum ferritin >800 ng/ml in 100% of patients with severe disease versus 30% in those with moderate disease, $p=0.003$). The difference could be due to a difference in the case selection. We included only patients with severe disease while 93% of survivors in the study conducted by Zhou *et al.*[1] had mild disease (CURB score 0–1) and 48% of the participants included by Chen *et al.*[12] were having non-severe disease.

There was a statistically significant increase in serum LDH level in non-survivors (from 816.2±443.08 U/L to 1056.61±480.54 U/L, $p=0.003$) as compared to survivors (from 829.59±499 U/L to 1018.6±468 U/L, $p=0.20$). According to a study from China[1], serum LDH level was significantly higher among non-survivors ($p<0.001$). Similarly, it has been reported that high LDH level is associated with Acute respiratory distress syndrome, admission to intensive care unit, progression of the disease and higher mortality.[1,9,21–23]

The D-Dimer level increased significantly in both survivors and non-survivors (from 7.2±9.8 µg/ml to 28.8±35.4 µg/ml, $p=0.01$ and from 8.75±14.8 µg/ml to 29.52±37.96 µg/ml, $p=0.001$, respectively). Published literature indicates that D-Dimer levels were significantly higher among those with severe disease.[1,5,7,10,21] This discrepancy may be due to the homogenous nature of our study population (severe COVID-19 patients admitted to ICU) while the majority of other studies had patients with mild, moderate and severe disease. It has been reported that patients with non-severe disease have a lower level of D-Dimer as compared to those with severe disease.[24]

Our sample was a homogenous one including only cases with severe disease admitted to intensive care unit. To the best of our knowledge, this is the first study on the role of biomarkers in COVID-19 patients admitted to intensive care unit. It had a relatively small sample size and it was a single-center experience.

CONCLUSION

Serum CRP and LDH levels predict mortality in patients with severe COVID-19 and can be used for risk stratification. Lymphopenia, increase in serum ferritin and D-dimers are frequently observed in patients with severe COVID-19 but these may not predict survival. Clinicians caring for patients with COVID-19 should use serum CRP and LDH levels for risk stratification and prognosis.

Conflicts of interest

All authors declare no conflicts of interest.

AUTHORS' CONTRIBUTION

MN, SA, FR: Concept. SB, HG, AM, MU: Literature search. HG, AM, RU, MAK: Data collection. SB, KS: Data analysis. SB, MN, SA, FR: Data interpretation. SB, MN, SA, FR, MU: Manuscript writing.

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