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Original Article

Biochemistry Section

Association of Serum Lactate Dehydrogenase and Qualitative C-Reactive Protein with the Severity of COVID-19 Disease

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ABSTRACT

Introduction: After December 2019, the word "COVID" became the nightmare to the civilisation. As per the nomenclature laid by World Health Organisation (WHO), the disease is called Coronavirus Disease-2019 (COVID-19) and the causative virus is Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2). By August 11th 2021, the virus caused around 43 lac deaths with an infection burden of approximately 20.3 crore cases worldwide. Many studies are published from most of the corners of the world regarding clinical features, laboratory parameters and radiological features of the disease to identify the infection at an early stage. Serum Lactate Dehydrogenase (LDH) and C-Reactive Protein (CRP) are among the most commonly studied parameters in COVID-19, though in India, a smaller number of studies were done in this regard. As the disease itself is new to the medical fraternity, maximum studies were done with small sample size which requires more studies to confirm the findings.

Aim: To find out the association of on-admission serum LDH and qualitative CRP with the severity of COVID-19 disease.

Materials and Methods: The present study was a retrospective observational study conducted for three months from May to July 2021. A 114 Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) positive COVID-19 patients were included as per the inclusion-exclusion criteria of which 57 were from Intensive Care Unit (ICU), considered as 'severe' patients and 57 from ward, taken as 'non severe' patients. Required blood parameters including LDH and CRP values were obtained from Laboratory Information System (LIS) and clinical data was obtained from hospital database. The values were analysed using statistical software.

Results: Present study showed significant difference in values of LDH among ICU and ward patients (p=0.0001), also significant difference of CRP positive percentage between these two groups (p=0.0003) was observed.

Conclusion: On the basis of the findings of the present study, it can be concluded that on-admission LDH and CRP can be used as a marker of severity in COVID-19 disease.

Keywords: Coronavirus disease-2019, Multiorgan dysfunction syndrome, Severe acute respiratory syndrome coronavirus-2

INTRODUCTION

Since more than a year, the whole world is fighting a pandemic of COVID-19. It was first observed in Wuhan, China in December 2019 as a contagious respiratory tract infection which in severe form causes atypical pneumonia and subsequently Acute Respiratory Distress Syndrome (ARDS) and lastly Multiorgan Dysfunction Syndrome (MODS) and death [1]. Later, the culprit virus was identified as SARS-CoV-2 [2]. As per WHO nomenclature, the disease was named as COVID-19 [3]. The disease spread to the rest of the world within a short span of time. On 30th January 2020, WHO declared it as a 'public health emergency of international concern [4] as the disease was spreading to rest of the world at a jet speed and ultimately on 11th March 2020, WHO announced COVID-19 disease as a global pandemic [3]. India is also fighting since January 30, 2020 when the first case of COVID-19 disease was identified in Kerala, India [5]. Presently, India has a total number of 3.2 crore cases with a death toll of 4.28 lacs [6,7]. The state of Assam is having a disease burden of 580,657 with number of deaths 5,502 [8]. (All data as on 11th August, 2021).

The most studied haematological parameters in COVID-19 infection are serum LDH, CRP, ferritin, D-dimer, lymphocyte count, Interleukin-6 (IL-6) etc. LDH is an enzyme which causes reversible conversion of pyruvate to lactate [9]. LDH is found in almost all the major organ tissues in abundance in the form of five different iso-enzymes like LDH1 is mostly found in heart, LDH2 in reticulo-endothelial system, LDH3 in lung, LDH4 in kidney and pancreas and LDH5 is abundantly found in liver and skeletal muscle. So, any tissue injury of these organs causes cell death and subsequent rise in serum LDH level [10].

The SARS-CoV-2 virus has higher affinity for Angiotensin Converting Enzyme 2 (ACE-2) receptors which are highly expressed on alveolar cell type 1 and type 2. The virus enters first through lung as a result of interaction between S protein of virus and ACE-2 receptor [11]. Gradually, it stimulates the macrophages in alveoli and then there is a stimulation of innate immunity which later becomes uncontrolled due to release of excessive pro-inflammatory cytokines [12], there is loss of alveolar-capillary barrier and pneumonia develops which turns to ARDS. As a result of lung tissue damage, LDH3 rises initially which reflects an increase in serum LDH level. In advance stage of the disease, further serum LDH rise occurs due to the injury of myocardium, kidney, liver etc., by the virus as a part of cytokine storm which causes MODS [13].

The CRP is used in the clinical practice from a long time as a marker of some pro-inflammatory conditions like sepsis. It is an acute phase protein in the pentraxin family of ligand-binding plasma proteins which are also calcium dependent [14]. Site of production is usually liver and it grossly responds to the tissue damage secondary to inflammation while stimulated by some pro-inflammatory cytokines like IL-6. In pro-inflammatory conditions it starts to rise within 4-6 hours and reaches its peak in 36-50 hours [15].

In COVID-19 disease, it is the cytokine storm which makes the disease complicated and severe. In cytokine storm, there is uncontrolled release of pro-inflammatory cytokines like IL-6 which ultimately leads to tissue damage and MODS. Keeping pace with

the extent of inflammatory process and tissue damage, CRP also rises in proportion. This makes CRP an important parameter to assess the disease prognosis and severity in COVID-19 disease as described in many studies in regard to COVID-19 disease [16,17].

The sheer number of patients being admitted with COVID-19 infection overwhelms the capacity to test for multiple parameters and risk stratify them. Many hospitals in India do not have the facility of conducting IL-6, ferritin or D-dimer tests. With this view, in present study, authors have included serum LDH and CRP, measured on admission, as a main variable with an aim to observe any association of these two parameters with the severity of the disease as in India comparatively less number of studies was being conducted in this regard. This will verify the early positive results of other countries and also will help to identify potentially high risk patients for developing severe disease so that timely intervention can reduce the mortality too.

MATERIALS AND METHODS

The present study was a retrospective observational study conducted for three months, in the Department of Biochemistry; Silchar Medical College, Assam, India. Data was collected from May 2021 to July 2021, and was analysed in August 2021. The study was done abiding all the ethical norms of the Institute. None of the identifications of the patients were disclosed. As it was a retrospective study, no patient was examined by the authors. Though, all the procedures were done as per the guidelines of the Ethical Committee of the Institute, but during the peak of the COVID-19 second wave here, ethical committee clearance could not be obtained, however considering importance of the situation and the need of the study, the study was proceeded further.

Inclusion criteria: Patients with age more than 18 years, patients diagnosed with COVID-19 infection by RT-PCR method and admitted in either the ward or the ICU were included.

Exclusion criteria: Patients diagnosed with haematological malignancies or solid tumours, patients taking immunosuppressive drugs for another disease, patients with a recent history of solid organ transplant or bone marrow transplant, patients with any known chronic disease of liver, kidney and heart, patients with disease of musculoskeletal system, patients with chronic systemic inflammatory disease.

Sample size calculation: The population was around 160 (since during the study period approximately 160 on an average COVID-19 patients were admitted daily) So, with 5% marginal errors and 95% Confidence Interval (CI), the sample size was calculated using online sample size calculator and it came around 113 [18]. The final sample size was taken as 114 for present study.

Data Collection

Total of 114 patients, who fulfilled inclusion-exclusion criteria; diagnosed for COVID-19 disease by RT-PCR method were included in the study. Among them 57 patients were included from COVID ward and 57 other patients were included from COVID ICU. As per the hospital protocol, clinically non severe patients (asymptomatic, mild symptomatic or patients who required less than 5 litre/min oxygen only and without any co-morbidity) were kept in COVID ward and in the present study, this group of patients was considered as non severe group. Clinically, severe patients (symptomatic with oxygen requirement more than 5 litre/min and with co-morbidities, patients who required invasive or non invasive respiratory assistance etc.,) were kept in ICU and this group was considered as severe group. Data regarding their biochemical parameters were extracted from the LIS of the Institute.

The values of serum LDH and CRP on admission to ward/ICU were collected for each patient. Cut-off CRP level was taken as 10 mg/dL [15]. Relevant history of the patient was collected from hospital database.

STATISTICAL ANALYSIS

Microsoft excel version 14.0.4734.1000 with add-ons and Graph pad online free version were used for statistical analysis. Mean and Standard Deviation (SD) was used to represent continuous variables whereas percentage used for categorical variables. Analysis regarding any association of serum LDH and CRP with the severity of disease was done by comparing serum LDH level and qualitative CRP in both the groups. Among statistical tests, Chi-square statistics were used where $p<0.05$ was taken as significant and unpaired t-test with CI of 95% was also used.

RESULTS

Data of 114 COVID patients were analysed (57 from ICU and 57 from ward). Distribution of study participants according to the age showed around 43.9% of the patients falls in the age group of 40-59 years. In ward and ICU also, this age group 40-59 years had the maximum number of patients with percentage of 42.1% and 45.6%, respectively. The mean age of the ward patients was 51.5 years where as in ICU patients, mean age was 58.73 years [Table/Fig-1].

Age groups (in years)	Frequency n (%)			Mean±SD (in years)		
	All (n=114)	ICU (n=57)	Ward (n=57)	All (n=114)	ICU (n=57)	Ward (n=57)
18-39	18 (15.8)	04 (7)	14 (24.6)	55.22±14.18	58.73±13.46	51.55±14.16
40-59	50 (43.9)	26 (45.6)	24 (42.1)			
60-79	40 (35.1)	21 (36.9)	19 (33.3)			
>80	06 (5.2)	06 (10.5)	0			

[Table/Fig-1]: Distribution of the study participants according to age (n=114).

Gender wise distribution shows male patients were 64.9% of total. The sex distribution was not significant statistically between ICU and ward patients (severe and non severe patients) with p-value of 0.6946 [Table/Fig-2].

Gender	Total participants N (%)	COVID-19 ICU participants N (%)	COVID-19 ward participants N (%)	p-value
Males	74 (64.9)	36 (63.2)	38 (66.6)	0.6946
Females	40 (35.1)	21 (36.8)	19 (33.4)	

[Table/Fig-2]: Details of gender analysis.
Chi square statistical test

It was found that 58.7% patients were CRP positive of which 75.4% ICU patients had positive CRP and 42.1% ward patients had positive CRP. The difference was statistically significant with p-value of 0.0003 [Table/Fig-3].

Variable	Total participants n (%)	ICU participants n (%)	Ward participants n (%)	Statistical significance between two group
CRP	Frequency	Frequency	Frequency	p-value
Positive	67 (58.7)	43 (75.4)	24 (42.1)	0.0003
Negative	47 (41.3)	14 (24.6)	33 (57.9)	

[Table/Fig-3]: Details of CRP analysis.
Chi square statistical test

The values of serum LDH level between severe COVID-19 patients (ICU patients) and non severe COVID-19 patients were compared (ward patients), the difference came out extremely significant with p-value of 0.0001 [Table/Fig-4].

Blood parameter	Mean±SD (n=114)	Mean±SD		p-value
		ICU (n=57)	Ward (n=57)	
LDH	551.69±313.79	660.41±377.44	441.04±175.34	0.0001

[Table/Fig-4]: Comparison of serum LDH between ICU and ward patients.
Unpaired "t" test

DISCUSSION

The clinical spectrum of COVID-19 infection can vary from asymptomatic forms to interstitial pneumonia with different lung damage and the development of ARDS [19]. In COVID-19 patients, LDH and CRP might represent an expression of lung damage and might reflect the respiratory distress consequent to the abnormal inflammation status. In a small cohort of 27 patients, CRP correlated with CT findings and resulted significantly increased at the early stage of severe COVID-19 before changes in the CT score [20].

In the present study, most patients were in the age group of 40-59 years with a percentage of 43.9% and there was a male dominancy with 64.9%. Wu MY et al., in their study observed 47 patients were male out of 87 (54%) and their median age was 44 years [9]. Zhang ZL et al., in their systemic review with 28 studies and meta-analysis with seven studies which comprises of 4663 patients observed that the mean age of the studies was 48.4 years with 46.7% female participants [2].

In the present study, 43 CRP positive cases were out of 57 ICU admitted patients (75.4%), and 24 CRP positive cases out of 57 non ICU patients (42.1%). The authors got the LDH mean of 551.69 U/L (with range of 182.8-2115.4 U/L) and in non severe patient group it was 441.04 U/L and in severe group 660.41 U/L. So, there was statistical significance of the difference of both CRP and LDH (p-value is 0.0003 and 0.0001, respectively) in severe group when comparing with the non severe group. Wu MY et al., got LDH level 495.1±28.22 U/L with a range 158-1482 U/L in 87 patients while in non severe group they found the level was 442±17.47 U/L and in severe group 1040±158.3 U/L. They found the difference was statistically significant with p-value of <0.01 [9]. Zhang ZL et al., found both the CRP and serum LDH was high in severe group of patients in most of the study [2]. Fan BE et al., in their analysis, which was done with COVID patients, ICU (n=9) and non ICU (n=58) patients got significant difference of LDH level between two groups (p-value=0.005) [4]. Though this study comprised of small sample size but the finding was similar to the present study which has comparatively large sample size with COVID ICU (n=57) and non ICU (n=57) patients with statistically significant serum LDH difference (p<0.0001).

Tjahyadi RM et al., in their study with 69 COVID-19 patients found that, in 37 patients of severe group CRP value was significantly high compare to mild to moderate group and they found the association of CRP with the disease severity with p-value=0.011 [17]. They also got higher serum LDH value in severe group with a mean of 1047 U/L (range 524 U/L-2239 U/L) compared to mild to moderate group which was 717.35 U/L as mean (range 270 U/L-1570 U/L). They found the difference was statistically significant with p-value<0.001 [17]. The result was very similar to the finding of the present study where both the CRP and serum LDH which was measured on-admission, have statistically significant difference in values in ICU group of patients compared to ward-patient group with a p-value of 0.0003 and 0.0001, respectively. So, many studies showed positive correlations between LDH and severity of the disease and between CRP and the disease severity and support the findings of the present study.

Limitation(s)

The present study had some limitation. Firstly, larger sample size is required for the validation of the findings and secondly, the authors didn't consider super-added infections or sepsis which may appear secondarily and can influence these two parameters.

CONCLUSION(S)

Based on the findings of the present study, it can be said that as the on-admission serum LDH and CRP were found significantly higher in severe groups of COVID-19 patients (ICU patients), these two parameters can be used as markers of severity in SARS-CoV-2 infection and can be used to identify the potentially high risk patients who may develop severe form of the disease. This may help in decision making in case of high risk patients and thereby will help to reduce the mortality and morbidity. Moreover, the result of the present study has verified the findings of other studies where these two parameters were found to be clinically significant.

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