

ORIGINAL RESEARCH**Comparison of LDH-to-lymphocyte ratio in critical and non-critical COVID-19 patients and assessment of its utility in predicting the severity of infection****Manidip Chakraborty^{1*}, Kaushik Tripura², Jayanta Debnath^{3*}, Biswadeep Choudhury⁴**¹Senior resident, Department of Biochemistry, Tripura Medical College and Dr.BRAM Teaching Hospital, Agartala, Tripura, India.²Associate professor, Department of Community Medicine, Tripura Medical College and Dr.BRAM Teaching Hospital, Agartala, Tripura, India.^{3*}Associate professor, Department of Microbiology, Tripura Medical College and Dr.BRAM Teaching Hospital, Agartala, Tripura, India.⁴Professor, Department of Biochemistry, Silchar Medical College and Hospital, Cachar, Assam, India.**Corresponding author:**Dr. Jayanta Debnath, Department of Microbiology, Tripura Medical College and Dr.BRAM Teaching Hospital, Agartala, Tripura, India, Email: drjdebnath@gmail.com**Received: 2-11-2022****Revised: 24-12-2022****Accepted: 26-12-2022****ABSTRACT:**

Background: It is a fact that the ongoing worst ever pandemic caused by Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2), have affected millions of people with large number of casualties worldwide. There has been now several published studies regarding the clinical, laboratory and radiological findings of COVID 19 infections. The aim of this study was to combine the two common hematological abnormalities viz. lymphopenia and elevated LDH to increase the sensitivity of diagnosis of COVID-19 infection. We compared the LDH-to-lymphocyte ratio in patients with COVID-19 infection admitted in the ICU and general ward to assess its relation with severity of disease. Our study may help in early identification and categorization of COVID-19 patients.

Methodology : A total of 100 adult patients diagnosed by RT-PCR for COVID-19 were recruited for the study. One patient was excluded due to unclear history. We included 49 patients with severe disease from intensive care unit (ICU) and 50 patients from COVID ward with less severe disease. Serum lactate dehydrogenase (LDH) levels and absolute lymphocyte counts (ALC) of these patients were obtained during admission and the ratio of LDH to ALC was calculated for both groups. The collected data was statistically analyzed.

Results : Serum LDH levels were higher in ICU patients group compared to the COVID ward patients. ALC on admission were lower in ICU patients than the ward patients. In both cases the difference was statistically significant with p value of 0.001 in all the cases. A p value of <0.05 was considered as statistically significant. LDH/ALC ratio median of all included patients was 0.390 with interquartile range (IQR) 0.450. In COVID ICU group the median of the LDH/ALC ratio was 0.580 with IQR 0.340 and in COVID ward group median was 0.220 with IQR 0.240. It suggests that the LDH/ALC ratio is significantly higher (p value of 0.001) in COVID ICU patients with severe disease in comparison to COVID ward patients having non-severe disease.

Conclusion: LDH/ALC ratio on admission can be used as a sensitive prognostic marker in COVID-19 patients which can help in early identification of COVID patients who have potential risk to develop severe disease.

Keywords: Lactate dehydrogenase (LDH), Absolute lymphocyte count (ALC), COVID-19, LDH/ALC ratio.

INTRODUCTION:

On 31st December 2019¹, People's Republic of China reported to WHO regarding one very infectious viral respiratory tract infection in Wuhan, Hubei province which often turns into atypical pneumonia and lastly acute respiratory distress syndrome (ARDS) and multi organ failure leading to death, especially in immune-compromised host. WHO declared it as a "public health emergency of international concern" on 30th January 2020⁵ and on March 2020 WHO declared it as "pandemic"⁶. It has become the greatest pandemic ever on Earth affecting more than 180 countries. The causative organism was identified as human novel corona virus also known as severe acute respiratory syndrome corona virus 2 (SARS-COV-2)⁷. As per WHO nomenclature the disease was termed as corona virus disease 19 or COVID-19. As on December, 2022 there has been 624 million confirmed cases and over 6.5 million deaths worldwide³. India harbours 44.6 million diagnosed case burden and around 0.53 million casualties⁴.

The diagnosis of COVID-19 infection heavily relies on molecular tools like RT-PCR. The two approaches adopted worldwide target specific genes in the viral genome. The first approach is approved by WHO and targets the E, RdRp and N genes while the second approach approved by CDC (USA) targets N1/2/3 gene and uses RNase P gene as a control assay. The widespread implementation of these methods led to the identification of many clinically silent cases in the community and strengthened the measures to limit the spread of infection. However, it was soon realized that RT-PCR based methods have their own limitations and can lead to false-negative as well as false-positive results.⁹

Based on the previous experiences of SARS-COV-1 and MERS-COV, many parameters among the hematological investigations have been implicated as prognostic markers in COVID-19 while a few have been studied as diagnostic markers. The most common finding among patients of COVID-19 has been found to be decrease in the absolute lymphocyte count (ALC) $< 1.0 \times 10^9/L$ i.e. lymphopenia. A recent meta-analysis noted that 35-75% patients developed lymphopenia and was more common in those who died of the disease.¹⁰ This lymphopenia has been thought to represent an impaired immune response to the infection. It has been found that COVID-19 reduces the CD4+ subset of lymphocytes and leads to higher naïve CD4+ cells than the memory cells. This increased naïve: memory cell ratio is a marker of severe impairment in immune response.¹¹ Other important hematological abnormalities found are leukocytosis (lymphocytosis/neutrophilia) suggestive of cytokine storm or superadded infection, thrombocytopenia, increased markers of systemic inflammation like C-reactive protein (CRP) and deranged parameters of coagulation like prothrombin time (PT), activated partial thromboplastin time (aPTT) and D-dimer.⁶

Lactate dehydrogenase (LDH) is an enzyme found in multiple organ systems and plays a role in the interconversion of pyruvate and lactate at the sub-cellular level. It is present in five different isoforms in our body viz. LDH1 in heart, LDH2 in reticuloendothelial system, LDH3 in lungs, LDH4 in kidneys and pancreas and LDH5 in liver and skeletal muscle. Apart from these major organ systems, LDH can be found in virtually every tissue of our body. As a result, injury to any of these organs may lead to release of LDH and rise in its levels in the blood. COVID-19 infection of the lungs, similarly, leads to elevated LDH levels representing injury primarily to the lungs as a result of pneumonia. Apart from pneumonia, elevated LDH can be found in COVID-19 as a result of myocardial injury, acute kidney injury (AKI), liver injury and as a part of the cytokine storm leading to MODS.¹²

A recent meta-analysis showed that elevated LDH is associated with worse outcomes in COVID-19 patients. There was a > 6 -fold increase in odds of severe disease and >16 -fold increase in odds of mortality in patients with elevated LDH. Thus, it can be used as an important tool in determining the prognosis in COVID-19 infection.¹³ The aim of this study was to combine the two common hematological abnormalities viz. lymphopenia and elevated LDH to increase the sensitivity of diagnosis of COVID-19 infection. We also intend to compare the LDH-to-lymphocyte ratio in patients with COVID-19 infection admitted in the ICU and general ward and assess its relation to severity of disease. Numerous studies regarding the clinical course, prognosis of the disease have been published worldwide. All most all the studies suggested that mortality is higher in group of patients with severe disease. Our study aims to investigate the patients of COVID-19 disease at an early stage who may proceed towards severe form of the disease so that early intervention in this regard can be done to minimize the mortality and morbidity.

METHODS:

This was a retrospective observational study conducted in a tertiary care teaching hospital of Assam, North East India from June 2020 to December 2020. After due permission from the Institutional Ethics Committee, hundred patients of COVID-19 disease were included in the study who were diagnosed through RT-PCR method. One of them was excluded from the study later on due to unclear history. Among them, fifty patients were included from COVID ward where less severe and mild patients were kept and forty nine patients were included from COVID intensive care unit (ICU) where patients with severe disease were kept. All the patients were included in this study after considering the inclusion-exclusion criteria as mentioned below.

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.

Exclusion criteria:

- Patients diagnosed with hematological malignancies or solid tumors.
- Patients taking immunosuppressive drugs for another disease.
- Patients with a recent history of solid organ transplant or bone marrow transplant.

Collection of data : Data regarding their hematological and biochemical parameters were extracted from the Laboratory Information System (LIS) of the institute. The required history of the patients was collected from database.

Analysis of data : The values of LDH and ALC on admission to ward or ICU were collected and LDH/ALC ratio was calculated for each patient. The ratio was compared between COVID-ICU and COVID-ward patients.

Statistical analysis : Microsoft excel 2019 with add-ons was used for statistical analysis. Mean \pm standard deviation (SD) was used to represent continuous variables whereas percentage was used for categorical variables. Shapiro – Wilk and Leven’s test has been done to check for normality and homogeneity of data.

RESULTS :**Table no 1: Distribution of the study participants according to age (n=99)**

Age groups (in year)	Frequency	Percentage
15 – 30	13	13.1%
31 – 45	12	12.1%
46 – 65	51	51.5%
66 and above	23	23.2%

The mean age of the study participants was 54.8 years with a SD of 16.0. The frequency distribution of age groups shows that 51.5% of the patients fall in the age group of 46-65 years which seems to be in conformity with the commonly affected age group.

Table no 2: Distribution of the study participants according to gender (n=99)

Gender	Frequency	Percentage
Female	36	36.4%
Male	63	63.6%

Gender wise distribution shows 63.6% were male and 36.4% were female.

Table no 3:

Gender	Location		P value
	ICU	WARD	
Female	16 (44.4%)	20 (55.6%)	0.582*
Male	33 (52.4%)	30 (47.6%)	
Age	64 (18)	48 (23.5)	0.001**

* χ^2 test of continuity correction **Mann - Whitney U test, p value < 0.05 taken as statistically significant, Shapiro – Wilk and Leven’s test done to check for normality and homogeneity of data.

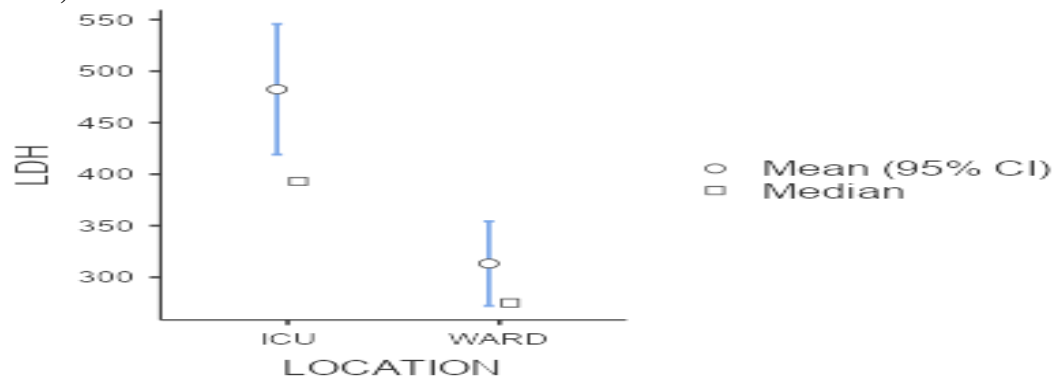
Gender wise difference in number of admission in COVID ICU and ward is not statistically significant as p value is 0.582 (p value <0.05 taken as statistically significant). But Age wise admission in COVID ICU is very significant. Among the COVID ICU patients, the mean age is more than the COVID ward patients and this difference is also statistically significant as p value is 0.001.

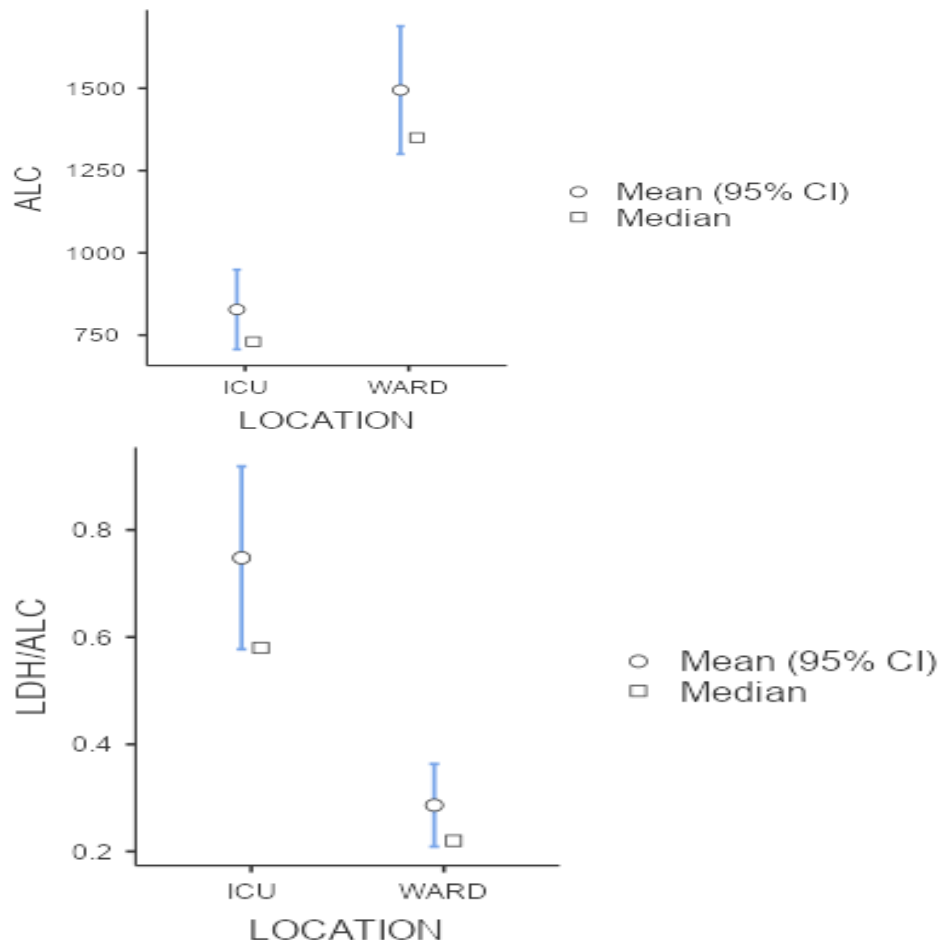
Table no 4: Descriptive statistics of different blood parameters

Blood parameters	Median (IQR) (n = 99)	Median (IQR)		p value*
		ICU (n=49)	WARD (n = 50)	
LDH	350 (204)	393 (180)	275 (112)	0.001
ALC	1030 (915)	730 (440)	1350 (868)	0.001
LDH/ALC	0.390 9 (0.450)	0.580 (0.340)	0.220 (0.240)	0.001

*Mann - Whitney U test, p value < 0.05 taken as statistically significant, Shapiro – Wilk and Leven’s test done to check for normality and homogeneity of data.

Table – 4 clearly shows that on admission, value of serum Lactate Dehydrogenase (LDH) is significantly higher in COVID ICU patients group compared to COVID ward patients group and the absolute lymphocyte count (ALC) is significantly lower in ICU patients than the ward patients. On calculation of the LDH/ALC ratio followed by statistical analysis, it reflects that there is distinctly high value of LDH/ALC ratio in COVID ICU patients in comparison to COVID ward patients. The difference is statistically significant with p value of 0.001 in all the cases. A p value of <0.05 is considered as statistically significant.

LDH, LDH/ALC



DISCUSSION:

Along with the pace of time after declaration of COVID-19 disease as pandemic by WHO, many studies have been published worldwide regarding the biochemical, hematological and radiological changes which helps in diagnosis and can be used to prognosticate the disease. Among these parameters studied, serum lactate dehydrogenase (LDH), ferritin, procalcitonin, plasma D-dimer, blood lymphocyte count etc. are the most important ones. But studies relating to extensive evaluation of serum LDH and blood lymphocyte count and on their ratio are comparatively less. Our study aimed to determine the ratio of serum LDH and absolute lymphocyte count (ALC) on admission, in relation to the severity of the disease for early identification of the potentially risky patients who may develop severe form of the disease.

In our study we found that on admission serum LDH was more in COVID ICU patients with severe disease compared to COVID ward patients with mild or less severe disease. The median was 393 with interquartile range (IQR) 180 in COVID ICU patients and in COVID ward patients median was 275 and IQR 112 (Table:4). We also observed that absolute lymphocyte count (ALC) on admission were lower in COVID ICU patients with a median of 730 with IQR 440 while comparing with the COVID ward patients with median 1350 and IQR 868. The median of ALC of all patients was 1030 with IQR 915.

Fan Eugene B et al. in their study named "Hematologic parameters in patients with COVID-19 infection" also found lower ALC in COVID ICU patients (median ALC $0.4 \times 10^9/L$) while they compared with non-ICU patients (median $1.2 \times 10^9/L$)¹. They also observed initial serum LDH level was higher in COVID ICU patients with a median value of 1684 U/L (reference value 270 U/L to 550 U/L)¹. Frater LJ et al. in their review article titled "COVID-19 and clinical hematology laboratory" also mentioned about lymphopenia in severe COVID patients in most of the studies they reviewed⁶. Wu M. et al. in their study named "Clinical evaluation of potential usefulness of serum lactate dehydrogenase (LDH) in 2019 novel

coronavirus (COVID-19) pneumonia” showed the significant difference in serum LDH level between severe and non-severe COVID patients (p value <0.05; where p value <0.05 was taken as significant). They also described that LDH level was proportionate to the radiological improvement of COVID pneumonia¹⁴. Henry B.M. et al in their pooled analysis named “Lactate dehydrogenase levels predict coronavirus disease 2019 (COVID-19) severity and mortality: A pooled analysis” found 84.6% of severe COVID patients were having raised serum LDH level compared to 43.3% in non-severe group¹³.

In our study, LDH/ALC ratio median of all included patients was 0.390 with IQR 0.450. In COVID ICU group the median of the LDH/ALC ratio was 0.580 with IQR 0.340 and in COVID ward group median was 0.220 with IQR 0.240 (Table : 4). It suggests that the LDH/ALC ratio is significantly higher (p value of 0.001) in COVID ICU patients with severe disease in comparison to COVID ward patients having non-severe disease.

In a retrospective observational study by Serin I et al. titled “A new parameter in COVID-19 pandemic: initial lactate dehydrogenase (LDH)/Lymphocyte ratio for diagnosis and mortality” with 2217 patients found 53% of their participants were male while rest of 47% were female and mean age was 47.66 ± 17.23 years¹⁵.

In our study we got 63.6% patients were male and 36.4% were female (Table: 2) but there was no significant relation based on gender between COVID ICU and COVID ward patients (p value 0.582 whereas p value of <0.05 was taken as significant)[Table: 3]. While analyzing the data regarding age of the patients we found that most of the patients belonged to the age group of 46 to 65 years which is around 51.5% (Table: 1). The median of age of COVID ICU patients was 64 years with IQR 18 while the median of age in COVID ward patients was 48 years with IQR 23.5. The difference between two was found statistically significant with p value of 0.001(Table: 3). It implies that with increase in age there can be higher possibility to develop severe disease.

Serin I et al. observed in their study that higher LDH/lymphocyte ratio bears the risk of higher mortality. They used involvement of lung in CT scan as gold standard to diagnose COVID to avoid false negative RTPCR results. They found the relation of LDH/lymphocyte ratio with the diagnosis of COVID as well as with the survival assessment¹⁵. Li G et al in their observational study titled “Lactate dehydrogenase-Lymphocyte ratio for predicting prognosis of severe covid-19 disease” with 145 patients found median age of their patients was 69 years with IQR 63.00. They observed, common complications of COVID were associated with patients with high LDH/lymphocyte ratio (p value <0.05). While they compared between survivors and non-survivors, they found non-survivors had significantly high LDH/lymphocyte ratio (p value <0.001)¹⁶. Similar to our findings, they also did not find any relation between gender and severity of COVID-19 disease. They concluded with the remark that LDH/lymphocyte ratio can be an independent prognostic factor in COVID-19 disease.

LIMITATION OF THE STUDY:

This study has some limitations like the LDH/ALC ratio in other diseases were not clear and the LDH/ALC ratio in other infections are also not well studied. So in mixed infection with COVID or even COVID with other pre-existing diseases this ratio may create confusion. More studies with large patient numbers are required to confirm the findings of this study.

CONCLUSION:

We conclude that on admission serum LDH and absolute lymphocyte count (ALC) can be used as an important prognostic marker of COVID patients and LDH/ALC ratio also can be determined to identify the patients who has potential risk to develop severe disease in order to plan early intervention to decrease the mortality and morbidity. Moreover age may be another factor related to severe form of the disease, of which age group of late forty to late sixty may be more susceptible.

CONFLICT OF INTEREST:

There was no conflict of interest.

FUNDINGS:

No funding was received for this study.

ACKNOWLEDGEMENT:

The authors sincerely thank Dr. Kushal Kalvit, Tata Memorial Hospital, Mumbai, Dr. Nabiha Mayanaz Karim, PGT Biochemistry, Silchar Medical College, for their supports. We are grateful to all the faculty members of Dept. of Biochemistry, Silchar Medical College, for their encouragement and lastly we convey out thanks to all the technicians of central composite laboratory for their help and support.

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