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Relationship of Viral Load with the Laboratory Markers and Prognosis in COVID-19 Patients

Zahra Ataee[1], Ali Rahmani fard[2], Saeid Amel Jamehdar[3], Majid Khadem-Rezaiyan[4], Maliheh Ziaee[5]*

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Abstract

Background: Coronavirus disease 2019 (COVID-19) viral load determined from the cycle threshold (CT) values may be a marker of disease severity and predict disease progression. Our study aimed to investigate the relationship between SARS-CoV-19 cycle thresholds or viral load, laboratory markers, and patient prognosis.

Methods: Patients who were admitted to Imam Reza Hospital at Mashhad University of Medical Sciences between March 2020 and March 2021 and had COVID-19 polymerase chain reaction (PCR)-confirmed at random were included in this cross-sectional study. Patients were randomly selected from those who tested positive on nasopharyngeal and oropharyngeal reverse transcription-PCR samples. The inclusion criteria were all patients older than 16 years with positive COVID-19 PCR results. Samples with Ct values of ≤36 were considered positive for SARS-CoV-2 RNA. Patients who did not have laboratory markers were excluded. We used SPSS Version 16 (Pearson correlation, analysis of variance, and logistic regression tests) to analyze the data. A $P \leq 0.05$ was considered statistically significant.

Results: In our study, serum lactate dehydrogenase and aspartate aminotransferase were found to be laboratory biomarkers inversely correlated with COVID-19 Ct values, indicating higher viral load (r = -0.14; $P = 0.024$ and r = -0.12; $P = 0.053$, respectively). Also, the platelet count is lower in patients with higher viral loads (r = 0.18; $P < 0.001$). However, we found no correlation between the viral load and some laboratory biomarkers such as ferritin, white blood cell and lymphocyte count, alanine transaminase, and c-reactive protein ($P > 0.05$). The patient's length of hospital stay was not correlated with their viral load ($P > 0.05$).

Conclusion: The COVID-19 viral load has been linked to some laboratory indicators and may be used to predict patient death. These discoveries might help in the treatment of COVID-19 disease.

Keywords: COVID-19, Biomarkers, Viral Load

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Introduction

Since its emergence in Wuhan, China, corona virus disease 2019 (COVID-19) has severely affected societal function (1). Millions of people died because of this condition and several million experienced the heavy compli-

Corresponding author: *Dr Maliheh Ziaee, ziaee.m@gmu.ac.ir*

1. Department of Internal Medicine, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
2. Mashhad University of Medical Sciences, Mashhad, Iran
3. Antimicrobial Resistance Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
4. Department of Community Medicine, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
5. Department of Community Medicine, School of Medicine, Social Determinants of Health Research Center, Gonabad University of Medical Sciences, Gonabad, Iran

↑What is "already known" in this topic:
Laboratory data for patient care have received minimal consideration in the COVID-19 disease treatment guidelines. The viral load of the virus can have an impact on laboratory findings and the prognosis of the disease. These findings suggest that early therapy initiation may result in successful treatment.

→What this article adds:
The results of the current study showed that lab results may alter the course and nature of a patient's care. The COVID-19 viral load has been linked to a number of laboratory indicators, and it has the potential to predict patient death. The findings might help in the treatment of the disease.

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cations of the virus. COVID-19 not only involves the lung, but also affects other systems such as the gastrointestinal, cardiovascular, and even central nervous systems (2). Although vaccination decreased the mortality rate, still mutations in the virus genome made it wilder, more infective, and even caused more severe symptoms (3). In addition to all of these issues, determining the disease's diagnosis is problematic because many infected patients could be asymptomatic and have self-limiting flu-like symptoms (4).

The World health organization (WHO) proposed that the detection of COVID-19 RNA in the nasopharyngeal and oropharyngeal swabs by polymerase chain reaction (PCR) is the standard method to diagnose the infection (5). It is thought that the virus can also be transmitted within the first 14 to 21 days after the onset of symptoms (6). However, controversies have remained in the case of the sensitivity and specificity of this diagnostic modality. The diagnostic value of PCR mostly relies on the viral load, usually characterized by the cycle threshold (Ct) (7).

The Ct is defined as the number of cycles required for the fluorescent signal to cross the threshold (ie, exceeds the background level) (8). Ct levels are inversely proportional to the amount of target nucleic acid in the sample (ie, the lower the Ct level, the greater the amount of target nucleic acid in the sample) (9). It is believed by some studies that the COVID-19 viral load, proposed by Ct values, can be a marker of disease severity and can predict a patient's outcomes (6, 10). Moreover, some studies have been conducted on the relationship between the COVID-19 Ct level and other clinical factors (7, 11). However, the number of these studies is limited and the evidence is not enough to conclude cases of controversies. Here, our study aims to assess the relationship of Ct values of SARS-CoV-19 or the viral load with the laboratory markers and prognosis in patients with COVID-19.

Methods

This cross-sectional study was conducted on patients admitted to Imam Reza Hospital in Mashhad University of Medical Sciences (MUMS) from March 2020 to March 2021. Imam Reza Hospital is the largest center among referral centers in Mashhad and is considered one of the largest general hospitals and a renowned center for education and treatment in Iran, with a total of 856 beds. This hospital is one of the most important hospitals in Mashhad in the COVID-19 pandemic. The ethical committee of Mashhad University of Medical Sciences confirmed the study protocol (ethical code: IR.MUMS.MEDICAL.REC.1400. 551).

Reverse transcription polymerase chain reaction (RT-PCR) samples from Imam Reza Hospital patients who tested positive for nasopharyngeal or oropharyngeal samples were selected at random. Specimens that were younger than 16 years old or for which no laboratory biomarker was registered were excluded. Specimens with Ct values of ≤36 were considered positive for SARS-CoV-2 RNA. Demographic data, length of hospital stay, laboratory findings—including white blood cell [WBC], lymphocyte count, platelets, CRP, lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine transaminase (ALT), and ferritin—and outcomes were recorded. Additionally examined and contrasted was the Ct value for patients who passed away, were dischargedfrom the hospital, or were admitted to the intensive care unit.

The sample size was calculated using the correlation coefficient of the CRP level and the Ct value according to the study by Liu et al (12). With a power of 80% and a significance level of 5%, the sample size was set at 134 patients, but our sample size was 373 patients to obtain a better evaluation. We were able to collect more samples than the calculated sample size, which was due to the relatively large number of COVID-19 patients hospitalized in this referral hospital. We think that the larger sample size is what accounts for the robustness of the findings in our study.

According to the cycle threshold, we classified the Ct value as "highly positive" when it was below the Ct mean and "low positive" when it was above the Ct mean (13).

Variables of severity in our study based on the national covid treatment protocol (December- 2020) are as follows:

Admission criteria in the ward (severe): dyspnea and chest pain and pressure in the chest with or without fever ≥38 degrees, oxygen saturation (O_2) between 90% and 93%.

The criteria for hospitalization in the intensive care unit (ICU) (very severe): rapid progression of respiratory symptoms, especially exacerbation of dyspnea, tachypnea with a respiratory rate >30, PaO_2/FIO_2 <300 mmHg, oxygen saturation(O_2) <90%, an increased gradient of A-a, involvement of >50% of the lung in the computed tomography scan.

To ensure high-quality reporting of observational studies, the Strengthening the Reporting of Observational Studies (STROBE) guidelines were used in this study (EQUATOR guidelines) (14).

Statistical Analysis

Categorical and continuous variables were presented as frequency (%) and mean ± standard deviation, respectively. The Pearson correlation coefficient was used to evaluate the correlation between the Ct and study variables. One-way analysis of variance (ANOVA) and Tukey-Kramer post multiple comparison tests were used to evaluate the significance of the differences between different outcomes. Also, we conducted logistic regression for the prediction of the Ct ratio. Dependent variables had 2 possible outcomes and the logistic regression assumption (independence of errors, linearity in the logit for continuous variables, absence of multicollinearity, and lack of strongly influential outliers) was checked out. A $P \leq 0.05$ was considered statistically significant. We used SPSS Version16 to analyze the data.

Results

A total of 373 laboratory-confirmed COVID-19 patients, with ages ranging from 16 to 101 years were enrolled. Most of our patients were men (60.1%). The length of hospital stay ranged from 1 to 51 days, and the range of Ct was 9 to 36. A total of 246 (64.4%) patients were dis-

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Table 1. Baseline characteristics data

Variable	Mean±SD or frequency (%)
Sex	
Male	243 (60.1)
Female	161 (39.9)
Age (years)	62.76±18.36
Length of hospital stay (days)	12.8±27.29
Outcome	
Discharged	246 (64.4)
ICU	12 (3.1)
Deceased	124 (32.5)
CT[1]	21.22 (6.3)

Abriviation: [1:] CT: Cycle threshold

Table 2. Laboratory findings, age and length of hospital stay and their correlation with CT value

Variable	Mean± SD	Median (IQR[€])	Correlation with CT[¥]	
			r*	*P*
Age (years)	62.76±18.36	65 (50-77)	-0.07	0.204
Length of hospital stay (days)	11.6±8.8	9 (5-16)	0.02	0. 63
Ferritin (ng/ml)	579.51±250.69		-0.07	0.386
WBC[α] count×10^9(cells/L)	10.18±5.57	8.7 (6.6-13)	-0.04	0.322
Lymphocyte %	1176.55±1436.74	892.5 (586.75-1246)	-0.03	0.611
Platelet count×10^9 (cells/L)	226.59±112.57	213.5 (142-288.25)	0. 18	0.001
CRP[B] (mg/L)	126.17±197.28	93.7 (47-155.3)	-0.04	0.536
LDH[£] (U/L)	753.06±387.36	669.5± (495.75-917.5)	-0.14	0.024
AST[©](IU/L)	57.51±116.62	37 (26-58)	-0.12	0.053
ALT[®] (IU/L)	48.93±72.99	34 (19-56)	-0.08	0.191

Abriviation: [€:] IQR: Inter Quartile Range; [¥:] CT: Cycle threshold; [α:] WBC: White Blood Cell; [B:] CPR: C - reactive protein; [£:] LDH: Lactate Dehydrogenase; [©:] AST: Aspartate Aminotransferase; [®:] ALT: Alanine Aminotransferase
*Pearson correlation coefficient

Table 3. Comparison of CT value according to patient's outcome

Outcome	CT value	P_1	P_2			
			Discharged	ICU	Deceased	Deceased in non-COVID-19 wards
Discharged	22.76±6.34	0.001	-	0.589	0.001	-
ICU	20.8±6.9		0.589	-	0.613	
Deceased	18.87±5.78		0.001	0.613	-	

P_1: *P*-value of comparison between outcomes using ANOVA.
P_2: *P*-value of comparison between two outcomes using post hoc test.

Table 4. Association between laboratory markers and CT values

variables	B	SE[€]	*P* value	OR[¥]	CI[*] (95%)
LDH	-0.003	0.001	0.022	0.99	1.001-1.008
outcome	-0.82	0.31	0.01	0.34	1.29 – 6.25

Abriviation: [€:] SE: Standard Error
[¥:] OR: Odds ratio
[*:] CI (95%): Confidence Interval 95%

charged, and 124 (32.5%) were deceased (Table 1).

We found an inverse correlation between age, ferritin, WBC, lymphocyte, CRP, LDH, AST, and ALT to the Ct; however, there was only a statistically significant correlation between LDH and AST to the CT value (r = -0.14; *P* = 0.024 and r = -0.12; *P* = 0.053, respectively).

LDH and platelet count both had strong indirect correlations with Ct values (r = 0.18; *P* < 0.001 and r = -0.14; *P* = 0.024, respectively) (Table 2).

Among the 3 different studies' outcomes after hospitalization of patients (discharge from the hospital, remaining in the ICU during our study, and death), the mean Ct value of the discharged patients had significantly higher Ct values compared with the deceased patients (*P* = 0.001). (Table 3).

We performed logistic regression to predict the Ct value by laboratory markers and patients' outcomes. We found that only LDH and outcome could accurately predict Ct values, meaning that as LDH increases, Ct levels decrease and disease severity rises. Also, in patients who died or were admitted to the ICU, the Ct level was reduced and the severity of the disease was higher (Table 4).

Discussion

Briefly, the cycle threshold is the point at which the thermal cycles are defined as the thermal cycles where the fluorescent signal is greater than the background fluorescence (15). This is a semi-quantitative measure that helps in the broad categorization of viral genetic material in patient samples after testing by RT PCR. A higher Ct value indicates a lower viral load and vice versa. Based on our study findings, the mean Ct value of the discharged patients had significantly higher Ct values compared with deceased patients and it seems that Ct values can be one of the criteria for the prognosis of hospitalized covid patients.

The current study reports the series of COVID-19 cycle

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threshold values of the 373 hospitalized patients with positive PCR tests and assesses the relationship of the viral load, measured by the Ct value, with the laboratory markers and prognosis in patients with COVID-19. Studies that give the quantification of COVID-19 in clinical specimens by reporting Ct values of RT-PCR are limited. The mean Ct value of COVID-19 in the current study was 21.22 and the overall viral Ct range of positive samples was 9 to 36. A large USA series analyzed 4428 RT–PCR positive samples and their overall viral Ct range of positive samples was 6.2 to 37.9 (16).

Platelet count was significantly associated with the Ct value in our study ($r = 0.18$; $P < 0.001$), which means that platelet count is lower with higher viral load. However, there was not any correlation between WBC count and viral load. This was in contrast to the systematic review, which considered leukocytosis as a poor prognostic factor and leukopenia as a better factor (17).

Lower Ct levels were associated with increased serum LDH levels ($r = -0.14$; $P = 0.024$). LDH is an enzyme found in the cells of most body tissues and increases after tissue damage. Consequently, an elevated serum LDH level occurs in numerous clinical conditions, such as hemolysis, malignancies, severe infections and sepsis, liver diseases, and many other diseases. An elevated serum LDH level also reflects tissue destruction, and interstitial pulmonary fibrosis is seen as an important prognostic marker for lung injury (18). Elevated serum LDH level is an important feature in COVID-19 patients and could be a predictive feature in COVID-19 patients. SohaibAsghar et al demonstrated the role of LDH level as the most potential biomarker in predicting the severity of COVID-19, and a study by Zheng F concluded that LDH level is an important prognostic factor (19).

ALT and AST are the enzymes that detect liver damage observed during COVID -19. In the current study, serum level AST was associated with viral load ($P = 0.053$), but interestingly ALT had no significant correlations. According to Zhang et al, 14% to 53% of patients have abnormal liver enzyme levels during disease progression (20).

Numerous studies have identified elevated levels of CRP as an important factor in poor prognosis. CRP is a nonspecific acute-phase reactant induced by IL-6 in the liver. However, in our study, we did not find any correlation between CRP level and viral load. However, numerous studies indicate that lymphopenia is an important prognostic factor. We found no correlation between ferritin and CT levels, nor between the length of hospital stay (days) and Ct level.

According the findings of this study, viral load could be considered a predictive factor for mortality. As shown in Table 3, deceased patients had lower mean Ct values during the course of the disease than discharged patients (18.87 ± 5.78 and 22.76 ± 6.34, respectively) ($P < 0.001$). This is consistent with a study conducted at Massachusetts General Hospital (21). However, Atique et al showed that disease progression correlated directly with viral load in patients with a Ct value between 21 and 30, whereas there was no significant correlation between viral load and disease progression in patients with a CT value <21 and >30 (22). In our study, the Ct value of patients admitted to the ICU was not significantly lower than that of patients discharged without an ICU admission. The reason for this may be due to the protocols for the ICU admission of patients.

Study Limitations

Complete data on clinical and laboratory parameters were not available for some of the patients.

Conclusion

In our study, serum LDH and AST levels were found to be a laboratory biomarker inversely correlated with COVID-19 Ct levels, indicating higher viral load. We also found that viral load can be considered a factor in predicting mortality. Patients with a greater viral load have a decreased platelet count. However, we found no correlation between viral load and some laboratory biomarkers such as ferritin, WBC and lymphocyte count, ALT, and CRP. The patient's length of hospital stay was not correlated with their viral load.

The COVID-19 viral load was associated with some laboratory indicators and might be used to predict patient mortality. These findings may be essential to the management of COVID-19 disease.

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Conflict of Interests

The authors declare that they have no competing interests.

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