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Cycle Threshold Values Predict COVID-19 Severity and Mortality but Are not Correlated with Laboratory Markers

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ABSTRACT

Many studies have evaluated the possible utility of cycle threshold (Ct) values as a predictor of Coronavirus disease 2019 (COVID-19) severity and patient outcome. Given the inconsistent results, we aimed to evaluate the association between severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) Ct values and disease severity, inflammatory markers, and outcomes in Iranian patients with COVID-19.

A retrospective study of 528 patients with COVID-19 hospitalized from September 2020 to October 2021 was conducted. Demographic, clinical, and laboratory data of patients were retrieved from electronic medical records. Ct values were analyzed as a continuous variable after subcategorizing into 3 groups: low (Ct values<20), medium (Ct values 20 to 30), and high (Ct values>30).

Of the 528 patients (45.1% female) aged 13 to 97 years, 109 patients had low Ct values, 312 patients had medium, and 107 patients had high Ct values. Patients with low Ct values were more likely to present with critical COVID-19, require invasive mechanical ventilation and develop complications such as acute respiratory distress syndrome and pneumonia. Furthermore, patients with low or medium Ct values were more likely to die compared to patients with high Ct values. Multivariate analysis showed that patients with low or medium Ct values were more likely to have severe COVID-19 compared with patients with high Ct values. The multivariate analysis also showed

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a higher risk of mortality in patients with low Ct values compared to patients with high Ct values, although this was not statistically significant.

Our findings revealed that Ct values were an independent predictor of COVID-19 severity. The risk of mortality was higher in patients with low Ct values. However, further investigation is needed to address the correlation between Ct values and inflammatory factors.

Keywords: COVID-19; Cycle threshold; Prognosis; Viral infections; Viral load

INTRODUCTION

Almost 3 years have passed since the outbreak of the novel coronavirus disease 2019 (COVID-19). From January 3, 2020, to June 9, 2022, 7,233,117 confirmed cases of COVID-19, with 141,342 deaths, have been reported in Iran. Studies revealed that COVID-19 severity is different among patients. Patients present various symptoms ranging from asymptomatic infection to mild, severe, and critical manifestations.[1,2] Although the effectiveness of some treatments is controversial,[3] significant progress has been made in identifying COVID-19-related inflammatory and laboratory markers. In this regard, several attempts have been made to find a reliable marker to predict the severity of COVID-19 so that it can be used in selecting the appropriate treatment and better management of patients with severe COVID-19.

Laboratory tests have also been utilized to assess COVID-19 severity and outcome.[4-6] Reverse transcriptase polymerase chain reaction (RT-PCR) has been used as the main test for the identification of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA in the upper respiratory tract specimens of patients with COVID-19.[7] The Ct value, also known as the threshold cycle value, corresponds to the cycle when the fluorescent signals intersect the threshold. Lower Ct values represent a higher viral load.[8] Although limited, a series of recent studies have demonstrated a link between Ct values in patients with COVID-19 mortality,[9,10] infectiousness,[11] and disease progression.[12]

Furthermore, several studies showed an association between Ct values and COVID-19 severity.[10,12,13] However, a systematic review revealed little to no difference in viral load in asymptomatic and presymptomatic patients with COVID-19.[14] Due to the inconclusive results, further studies have been recommended to verify the link between Ct values and poor outcomes in patients with COVID-19 and the possible utility of Ct values to predict the clinical course and disease prognosis in patients.[15]

Study results are less consistent regarding the correlation between Ct values and laboratory findings. Although a significant correlation has been identified between Ct values and some hematological and biochemical markers, including lymphocytes and Lactate dehydrogenase (LDH), for other markers such as C-reactive protein (CRP) and neutrophils, studies have failed to provide adequate proof.[13,16,17]

To the best of our knowledge, this is the first report evaluating the correlation between Ct values and disease severity, inflammatory markers, and outcomes in Iranian patients. The aim of this study is to assess whether SARS-CoV2 Ct values can predict disease severity, inflammatory markers, and outcomes in COVID-19 patients. The factors influencing the relationship between Ct values and clinical outcomes in patients have also been discussed. The findings of this study can shed light on the relationship between Ct values and COVID-19 severity and mortality, as well as inflammatory markers.

MATERIALS AND METHODS

Study Participants

In this retrospective observational study, data on hospitalized patients with laboratory-confirmed COVID-19 admitted to the referral hospitals for COVID-19 in Imam Khomeini and Shohadaye Tajrish hospitals in Tehran, Iran, were studied. The study period extended from September 2020 to October 2021.

Variables and Data Collection

The SARS-CoV-2 Ct values were obtained from COVID-19 reference laboratories. Then, the subjects were identified using a hospital registry database. A total of 528 records were retrieved from electronic medical records. A group of five colleagues collaborated in completing the questionnaire.

The retrieved information included demographic data, clinical symptoms, laboratory findings, comorbidities, and patient outcomes. All data collectors were trained to find out the method of data gathering accurately, and data abstraction was assessed qualitatively. The first recorded data were considered baseline. Patients were included with no restriction on disease onset. The day of beginning symptoms was considered as the date of disease onset. The duration of patients' symptoms was defined as the days after symptoms began upon admission to the hospital. Complications included acute respiratory distress syndrome (ARDS), pneumonia, and multiple organ dysfunction syndrome (MODS).

Demographics and general information include age, gender, ethnicity, date of admission, date of disease onset, date of patient discharge, duration of COVID-19 symptoms, smoking history, Ct values, and severity of COVID-19 (18). Comorbidities reported in patients were as follows: controlled and uncontrolled diabetes mellitus, cardiovascular diseases, hypertension, asthma, chronic obstructive pulmonary disease, obesity, chronic kidney, lung, and liver diseases, malignancy, and autoimmune diseases.

For all patients, the first recorded data of laboratory tests were considered. We evaluated the peripheral blood profile inflammatory and biochemical markers, including LDH, CRP, creatinine, Creatine kinase-MB (CK-MB), Creatine kinase (CPK), Alanine transaminase (ALT), Aspartate transaminase (AST), albumin, ferritin, D-dimer, and Erythrocyte sedimentation rate (ESR).

The patient presented symptoms of fever, self-reported sensation of fever, cough, chills, dyspnea, shortness of breath, hypoxia, poor appetite, general weakness, sore throat, diarrhea, nausea, vomiting, fatigue, chest pain, headache, myalgia, joint pain, dizziness, decreased level of consciousness, sputum production, anosmia, and abdominal pain.

Furthermore, information related to the patients' supportive care (intensive care unit admission, oxygen therapy, non-invasive and invasive mechanical ventilation, extracorporeal membrane oxygenation, type of hospitalization, in-hospital complications of COVID-19, length of stay in the hospital, and patient outcomes (i.e., discharge or in-hospital mortality) was collected.

Viral Nucleic Acid Extraction and Molecular Detection of SARS-CoV2 Using RT-PCR

Patients were confirmed to be infected with SARS-CoV-2 by real-time PCR testing of samples from the upper respiratory tract (throat and nasopharynx). Ct values<40 were considered positive. Viral RNA was extracted from the samples using the BehPerp Viral Nucleic Acid Extraction Kit (BehGene Biotechnology, Shiraz, Iran), according to the manufacturer's instructions. A real-time PCR kit (COVITECH, Tehran, Iran) was used to identify SARS-CoV2. The reaction mixture (20 µL) was as follows: master mix, primers and probes mix, RNase-free water, and isolated RNA. The tests were performed on a Bio-Rad CFX96 real-time detection system.

Statistical Analysis

Descriptive statistics were used to analyze the patients' demographic and baseline characteristics. Mean ± standard deviation (SD), and median were used to describe the distribution of continuous variables. Frequencies (n) and percentages (%) were used to describe the distribution of categorical variables.

The baseline characteristics and outcomes of patients were compared among patients grouped based on disease severity and Ct values. Patients were categorized based on the severity of symptoms into 4 groups: mild, moderate-to-severe, severe, and critical.[18] The relationship between Ct values and other variables was analyzed as a continuous variable after subcategorizing into 3 groups. Based on the Ct values, patients were grouped into 3 categories (low Ct values<20; medium Ct values 20–30; high Ct values >30).[19,20]

According to the data distribution, Pearson or Spearman tests were carried out to identify the correlation between Ct values and any of the variables. The chi-square test was used to examine the significant relationship between two qualitative variables. The multivariate logistic regression model was used to control the confounders related to disease severity, inflammatory factors, and patient outcomes. All statistical analysis was carried out using SPSS software version 22 (IBM, Armonk, NY, USA). A value of $p<0.05$ was considered statistically significant.

RESULTS

Demographics and Clinical Characteristics of Patients with COVID-19

A total of 528 COVID-19 patients with a defined Ct value admitted to Imam Khomeini and Shohadaye Tajrish hospitals were studied. The mean age was 57±17 (13–97 years), of which 238 (45.1%) were female. Fifty-seven (11.9%) of patients were smokers. Forty-seven (9.1%) patients had mild, 139 (27%) had moderate to severe, 237 (46.1%) had severe COVID-19, and 91 (17.7%) were critically ill. The median time of COVID-19 duration (the time from symptom onset to the date of sample collection) was 8 days. Median Ct values were 24.8 (range 11-36.2). The median length of hospital stay was 7 days (range 1–79 days). One hundred and twenty-seven (24.1%) patients were admitted to an intensive care unit (ICU). COVID-19 complications have been reported in 110 cases (21.7%). All-cause in-hospital mortality was 18.1% (n=95), and 430 (81.9%) patients were discharged.

With regards to comorbidities, 349 (66.1%) patients had comorbidities. The most common comorbidities were hypertension (n=186, 35.3%), diabetes mellitus (n=130, 24.7%), and heart disease (n=98, 18.6%), followed by autoimmune and rheumatologic disorders (n=85, 16.3%). The most common symptoms were cough (n=372, 70.6%) and dyspnea (n=309, 58.6%), followed by fever (n=283, 53.6%), general weakness (n=281, 53.2%) and myalgia (n=254, 48.1%). Lung abnormalities on chest imaging were reported in 337 (96.3%) of the patients. Table 1 summarizes the baseline demographic and clinical characteristics of the patients studied.

Patients with critical disease were more likely to have uncontrolled diabetes (p=0.048), dyspnea (p<0.001), shortness of breath (p<0.001), a decrease in oxygen saturation below 85% (p<0.001), and a decrease in consciousness level, (p<0.001). They were also more likely to require oxygen via invasive mechanical ventilation (p<0.001). Furthermore, more patients with critical disease developed COVID-19 complications, including ARDS, pneumonia, and MODS (p<0.001).

Patients' Characteristics, Clinical and Laboratory Findings Grouped by Ct Values

To evaluate whether Ct values can be used to predict COVID-19 severity, level of inflammatory factors, and outcomes of patients, Ct values of patients were assessed both as a continuous variable and also by categorization of the patients based on Ct values into 3 groups of low (Ct values <20), medium (Ct values from 20 to 30), and high (Ct values <30).

Of the 528 patients, 109 patients had low Ct values, 312 patients had medium, and 107 patients had high Ct values. Patients with low Ct values were older than patients with medium or high Ct values (p=0.016) and were more likely to present critical COVID-19 (p=0.006). 28.7% of patients with critical COVID-19 had low Ct values, compared to 15.6% with medium and 12.4% with high Ct values. The history of smoking was the same in patients grouped based on Ct values (p=0.260). Regarding comorbidities, there was no significant difference among patients with COVID-19 when grouped based on the Ct values (Table 2). Furthermore, patients with low Ct values were more likely to have decreased oxygen saturation (less than 85%), decreased levels of consciousness, fatigue, and tachycardia. Patients' characteristics grouped based on the Ct values are presented in Table 2.

The WBC count was higher in patients with high Ct values than in those with low or medium Ct values. Significant positive correlations were observed between Ct values and WBC and platelet counts (p=0.013, p=0.01, respectively). However, neutrophil count was reversely correlated with Ct values (p=0.014). There was no significant difference in lymphocyte or neutrophil counts among patients grouped based on Ct values. There was no difference in AST, ALT, ALP, ferritin, CRP, or ESR among patients grouped based on Ct values (Figure 1).

Patients' Outcomes Grouped by Ct Values

Patients with low Ct values were more likely to require invasive mechanical ventilation (p=0.001). Patients with low Ct values were also more likely to develop COVID-19 complications such as ARDS and pneumonia (Table 3). LOS and ICU admission rates did not differ among patients grouped based on Ct values. Patients with low or medium Ct values were more likely to die than those with high Ct values (p=0.015). The mean Ct value was lower in deceased patients (23.23±5) compared to discharged patients (24.74±5; p=0.011).

Table 1. Baseline demographic and clinical characteristics of patients with COVID-19.

Demographic Data		**Clinical Symptoms, n (%)**		
Total number	528	Fever	37℃ to 38℃	183 (34.7)
			38.1℃ to 40℃	55 (10.4)
			>40℃	45 (8.5)
Age, mean (SD) (years)	57 (17)	Oxygen saturation	<85%	166 (31.6)
			85% to 90%	148 (28.1)
			90% to 93%	94 (17.9)
			>93%	92 (17.5)
		Self-reported sensation of fever		219 (41.5)
Gender, n (%)		Chills		241 (45.6)
Female	238 (45.1)	Dyspnea		309 (58.6)
Male	290 (54.9)	Shortness of breath		101 (19.1)
Smoking history, n (%)		Cough		372 (70.6)
yes	57 (11.9)	Poor appetite		141 (26.8)
Comorbidities, n (%)		Generalized weakness		281 (53.2)
yes	349 (66.1)	Sore throat		49 (9.3)
Autoimmune disease	85 (16.3)	Diarrhea		68 (12.9)
Diabetes mellitus, controlled and uncontrolled	76 (14.4), 54 (10.3)	Nausea and vomiting		149 (28.2)
Hypertension	186 (35.3)	Chest pain		93 (17.6)
Chronic kidney disease	34 (6.5)	Headache		132 (25)
Chronic lung disease	30 (5.7)	Myalgia		254 (48.1)
Chronic liver disease	7 (1.3)	Arthralgia		19 (3.6)
Heart disease	98 (18.6)	Dizziness		57 (10.8)
Malignancy	37 (7)	Decreased level of consciousness		53 (10)
***Abnormal chest imaging, n(%)**		Sputum production		128 (24.2)
Yes	337 (96.3)	Anosmia		17 (3.2)
Ground glass opacity (GGO)	269 (91.8)	Dysgeusia		11 (2.1)
GGO plus Alveolar (mix)	12 (4.2)	Tachycardia		17 (3.2)
Multifocal patchy consolidation	17 (5.9)	Hemoptysis		35 (6.6)
other	39			
Clinical outcomes, n (%)		Nasal congestion		5 (0.9)
ICU admission	127 (24.1)	Abdominal pain		49 (9.3)
In hospital mortality	95 (18.1)	Fatigue		47 (8.9)
Discharged	430 (81.9)	**Supportive therapies,** n (%)		
COVID19 complications		Invasive ventilation		84 (16)
ARDS	69 (13.6)	Non-invasive ventilation		183 (36.6)
Pneumonia	85 (16.8)	ECMO support		3 (0.6)
MODS	16 (3.2)	**Hospital length of stay, median (range days)**		**7 (1–79)**

SD: standard deviation; ARDS: Acute respiratory distress syndrome; MODS: Multiple Organ Dysfunction Syndrome; ECMO: extracorporeal membrane oxygenation. *No data was available for 178 patients

Table 2. Patients' characteristics and presentation are grouped based on the Ct values.

Characteristic	Low Ct values (<20) (n=109)	Medium Ct values (20 to 30) (n=312)	High Ct values (>30) (n=107)	*p*
Age, n (mean±SD)	109 (60.93±16)	312 (56.3±17)	105 (54.5±17)	**0.016**
Sex, n (%)				0.183
Male	65 (59.6)	161 (51.6)	64 (59.8)	
Female	44 (40.4)	151 (48.4)	43 (40.2)	
COVID-19 severity, n (%)				**0.006**
Mild	9 (8.3)	29 (9.6)	9 (8.6)	
Moderate	22 (20.4)	77 (25.6)	40 (38.1)	
Severe	46 (42.6)	148 (49.2)	43 (41)	
Critical	31 (28.7)	47 (15.6)	13 (12.4)	
Comorbidities, n (%)				0.4
Diabetes mellitus				
Controlled, uncontrolled	17 (15.6), 14 (12.8)	43 (13.8), 28 (9)	16 (15.1), 12 (11.3)	0.8,0.4
Hypertension	41 (37.6)	110 (35.3)	35 (33)	0.7
Chronic kidney disease	9 (8.3)	16 (5.1)	9 (8.5)	0.3
Chronic lung disease	6 (5.5)	18 (5.8)	6 (5.6)	0.9
Chronic liver disease	3 (2.8)	3 (1)	1 (0.9)	0.3
Heart disease	20 (18.3)	58 (18.6)	20 (18.9)	0.9
Malignancy	9 (8.3)	24 (7.7)	4 (3.8)	0.3
Autoimmune disease	13 (12.1)	57 (18.3)	10 (14.1)	0.3
Lung abnormality, n (%)	65 (98)	209 (95)	63 (95)	0.570
Fever				0.125
37℃ to 38℃	35 (32.1)	102 (32.7)	46 (43)	
38.1℃ to 40℃	10 (9.2)	35 (11.2)	10 (9.3)	
>40℃	7 (6.4)	33 (10.6)	5 (4.7)	
Oxygen saturation				**0.017**
<85%	40 (36.7)	99 (31.8)	27 (25.5.)	
85% to 90%	30 (27.5)	95 (30.5)	23 (21.7)	
90% to 93%	18 (16.5)	57 (18.3)	19 (17.9)	
>93%	18 (16.5)	42 (13.5)	32 (30.2)	
Chills	54 (49.5)	147 (47.1)	40 (37.4)	0.246
Dyspnea	71 (65.1)	176 (56.4)	62 (58.5)	0.056
Shortness of breath	19 (17.4)	69 (22.1)	13 (12.1)	**0.022**
Cough	68 (63)	232 (74.4)	72 (67.3)	**0.015**
Poor appetite	28 (25.9)	81 (26)	32 (29.9)	0.122
Fatigue	12 (11)	24 (7.7)	11 (10.3)	**0.020**
Generalized weakness	53 (48.6)	176 (56.4)	52 (48.6)	**0.002**
Sore throat	7 (6.4)	28 (9)	14 (13.1)	**0.025**
Diarrhea	14 (12.8)	39 (12.5)	15 (14)	0.086
Nausea and vomiting	32 (29.4)	87 (27.9)	30 (28)	0.667
Chest pain	22 (20.2)	57 (18.3)	14 (13.1)	0.129
Headache	29 (26.6)	82 (26.3)	21 (19.6)	0.219

Myalgia	49 (45)	158 (50.6)	47 (43.9)	**0.031**
Arthralgia	2 (1.8)	12 (3.8)	5 (4.7)	**0.007**
Dizziness	13 (11.9)	37 (11.9)	7 (6.5)	0.145
Decreased level of consciousness	12 (11)	31 (9.9)	10 (9.3)	**0.001**
Sputum production	26 (23.9)	78 (25)	24 (22.4)	0.089
Anosmia	3 (2.8)	10 (3.2)	4 (3.7)	**0.009**
dysgeusia	3 (2.8)	5 (1.6)	3 (2.8)	**0.012**
Tachycardia	7 (6.4)	9 (2.9)	1 (0.9)	**0.000**
Hemoptysis	6 (5.5)	21 (6.7)	8 (7.5)	0.112
Nasal congestion	0 (0)	3 (1)	2 (1.9)	**0.004**
Abdominal pain	8 (7.3)	34 (11)	7 (6.5)	**0.012**

SD, standard deviation.

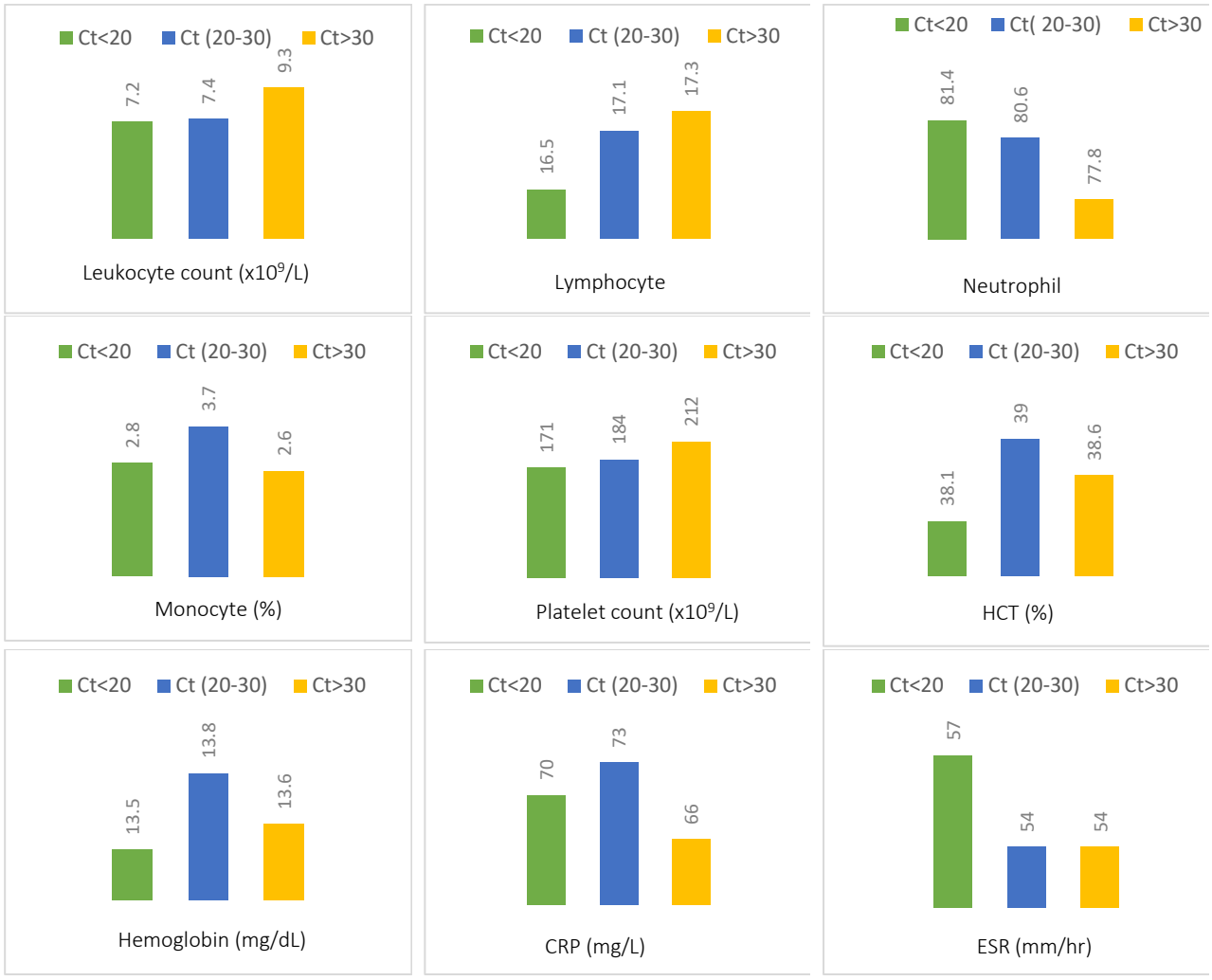

Figure 1. Laboratory findings in patients grouped based on Ct values. The levels of inflammatory factors and laboratory tests in patients with COVID-19 were compared in patients grouped according to Ct values. HCT, hematocrit; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; CPK, creatine phosphokinase; ALT, alanine transaminase; AST, aspartate transaminase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase.

Table 3. Length of hospital stay, COVID-19–associated morbidities, and outcomes of patients grouped by Ct values.

Characteristic	Low Ct values (Ct<20)	Medium Ct values (Ct 20-30)	High Ct values (Ct>30)	P value
Supportive therapy				
Invasive ventilation	28 (25)	48 (15)	8 (7)	**0.001**
Non-invasive ventilation	42 (38)	97 (31)	54 (50)	**0.002**
ECMO support	0	1 (0.3)	2 (1.9)	0.125
COVID-19 complication				
ARDS	22 (20)	41 (13.6)	6 (6.2)	**0.013**
Pneumonia	25 (23)	48 (15)	12 (12)	0.099
MODS	2 (1.9)	12 (4)	2 (2)	0.440
ICU admission	31 (28.7)	72 (23.2)	24 (22.4)	0.457
LOS (Day)	9 (8)	8 (5)	8 (6)	0.655
Death	29 (30)	53 (55)	13 (13)	**0.015**

ICU; intensive care unit; LOS; length of stay. ARDS: Acute respiratory distress syndrome; MODS: Multiple Organ Dysfunction Syndrome; ECMO: extracorporeal membrane oxygenation

Multivariate Analysis Adjusted for Age, COVID-19 Duration, and Other Risk Factors Related to COVID-19 Severity and Mortality

Multivariant analysis adjusted for age, COVID-19 duration, symptoms (fever, chills, dyspnea, shortness of breath, cough, level of O2 saturation, poor appetite, general weakness, sore throat, diarrhea, vomiting, nausea, chest pain, headache, myalgia, arthralgia, dizziness, decreased level of consciousness, sputum production, anosmia, dysgeusia, tachycardia, hemoptysis, nasal congestion, and abdominal pain), comorbidities (hypertension, controlled or uncontrolled diabetes, chronic kidney disease, chronic liver disease, chronic lung disease, heart diseases and malignancy), chest X-ray findings and oxygen requirement revealed that patients with low (<20) or medium (20 to 30) Ct values were more likely to have severe COVID-19 in comparison with the patients with high (>30) Ct values (p=0.041 and p=0.039, respectively). Increased risk of mortality was found in patients with low Ct values compared to those with high Ct values, although it was not statistically significant (p=0.091).

DISCUSSION

This retrospective study indicates a correlation between Ct values and COVID-19 severity even after adjusting for the confounding factors. Furthermore, after adjusting for other risk factors related to mortality, low Ct values correlated with an increased mortality rate, although it was not significant. Our findings are in concordance with other reports that have shown Ct values can be considered a predictive factor for COVID-19 severity and mortality.[10,20,21] Moreover, the results of a systematic review showed that the risk of mortality and severe COVID-19 was higher in hospitalized patients with Ct values<25 compared with patients with Ct values>30.[22]

There are inconsistent findings on this matter. A considerable amount of evidence shows that a high SARA-CoV-2 viral load positively correlated with COVID-19 severity.[10,12,13,21,23-25] Evaluation of the epidemiological characteristics of 10 patients with COVID-19 showed that Ct values were lower in severe patients compared to mild or ordinary patients (patients without severe COVID-19).[26] The results of another study revealed that Ct values were lower in critical patients with COVID-19 compared to the noncritical group, and Ct values <20 were associated with disease progression, development of ARDS, and requirement of mechanical ventilation.[27] A comparison of Ct values between symptomatic and asymptomatic cases in Germany showed lower Ct values in the symptomatic group.[28] An increase in the risk of respiratory failure was associated with Ct values<25 on admission in hospitalized patients in Spain.[29]

On the other hand, numerous studies have disproved the association of Ct values with COVID-19 severity.

No association was found between SARS-CoV-2 viral load and oxygen requirement in patients on admission .[30] Other studies have reported a lack of correlation between Ct values and disease severity in patients with COVID-19.[31-33] No difference in median Ct values was observed among the 4 symptom status groups of patients with COVID-19 (i.e., asymptomatic, presymptomatic, atypical symptoms, and typical symptoms).[34] Similarly, findings of two other studies showed that SARS-CoV-2 viral load was comparable between symptomatic and asymptomatic patients.[35,36] A study conducted in Brazil showed Ct values were also not different among hospitalized patients based on disease severity.[37]

The clinical relevance of SARS-CoV2 Ct values has been assessed in a cohort of patients with solid organ transplants. No significant difference was found between severe COVID-19 patients who required ICU care compared to other hospitalized patients.[38] In another study, Ct values were inversely correlated with the total severity score (TSS) of chest computed tomography scans in patients.[39] Ct values were also not associated with the disease severity, LOS, or requirement for ventilation in a cohort of patients with COVID-19 in a study conducted in Portugal.[40]

Another finding of our study was that low or medium Ct values in patients increased the risk of mortality. However, multivariate analysis showed no significant difference in mortality rate among patients grouped based on Ct values.

Some studies evaluated the association between Ct values or viral load and mortality.[37,41,42] Recently, a large retrospective study on 8318 patients with COVID-19 in the North of Iran showed that the rate of mortality was higher in patients with Ct values less than 20.[43] In another study on 1145 patients with COVID-19, the mean $\log_{10}$ viral load in deceased patients was higher compared to the surviving patients.[9] A high SARS-CoV-2 viral load was associated with an increase in the mortality rate in patients with or without cancer.[19]

Lower Ct values correlated with an increase in the mortality risk in another cohort of 1337 patients with COVID-19 in the Tayside region of Scotland, UK.[20] Furthermore, the risk of intubation and mortality rate were higher in patients with a high viral load (Ct <25).[44] The association between low Ct values and 30-day mortality rates has been reported in studies by Andrew Bryan et al,[45] and António Machado et al.[40] On the contrary, the results of some studies provided evidence against the association between Ct values and mortality rates.[33,39]

There is a wide spectrum of clinical and methodological diversity among the available studies that can be involved in this inconsistency. One reason for these controversial results is that different Ct cutoff values have been utilized. Furthermore, in some studies, Ct values have been evaluated as a continuous variable, while in others, they have been studied categorically. In a study by Ramamahesh Seeni et al., a range of Ct cutoff values has been used to evaluate the utility of Ct values as a predictor of the hospitalization requirement. Accordingly, a Ct value of 34 was found to be an appropriate Ct cutoff value to analyze all variables.[46]

In several studies, patients were grouped into 3 categories based on Ct values (<25, 25-30, and >30).[29, 41, 44] A study used a Ct cutoff value of 24 to compare mortality rates among patients grouped based on Ct values.[37]

In another study, the mortality rate among patients who had a high viral load (Ct <22) was compared to the patients with Ct values >22.[45] Ct values were divided into 4 groups: Q1 with Ct values <22.9, Q2 with values between 23.0 and 27.3, Q3 with values between 27.4 and 32.8, and Q4 with values >32.9 to analyze the relationship between CT values and patients' mortality .[42] In other studies, patients were grouped into 3 categories based on Ct values (<20, 20–30, and >30).[20, 30, 33, 38] Although, in most studies, Ct values <40 have been considered the cutoff, in a study by Ayşegul Karahasan Yagci, Ct values >40 have been used for analysis.[39] Another reason is that Ct values have been compared in patients with different clinical status. Some studies have compared Ct values between mild-to-moderate and severe forms of COVID-19.[10,12,21,23,24,47] However, Lee et al, made the comparison between symptomatic and asymptomatic patients.[36]

Factors found to be influencing COVID-19 severity and mortality have been investigated in several studies. Age and sex have been identified as predictive factors for mortality in COVID-19 patients.[20, 42] In our study, gender was not associated with COVID-19 severity. Notably, patients grouped based on Ct values showed no difference between men and women. These results are consistent with the findings of other studies.[19, 32, 48] Results of the current study also show that patients with critical COVID-19 were older than patients with mild or moderate COVID-19. Grouping of patients based on Ct values showed an association between age and Ct values; patients with low Ct values (<20) were older than patients with medium or high Ct values. Our findings are

comparable to the results of a study by Ramamahesh Seeni et al,[46] and Helena C. Maltezou et al.[41] In contrast to our finding, Xi He et al,[32] and Klinger Soares Fa´ıco-Filho et al, [37] argued that Ct values were not associated with age. Similarly, Fengjuan Shi et al, noted no significant differences in Ct values among patients in different age groups.[48] Carolin Ade et al,[49] and Yanan Zhao et al,[50] reached different results regarding the association of Ct values with age in patients older than 80.

Preliminary studies have shown an association between several comorbidities and COVID-19 severity or mortality. In our cohort of patients, we found no association between Ct values and comorbidities, which was inconsistent with the findings of other studies.[43,46] Patients grouped based on Ct values varied in terms of some comorbidities, such as cardiovascular disease, hypertension, kidney diseases, malignancy, and blood disorders.[43] Lower Ct values were reported in patients with diabetes mellitus and cardiac disease.[46]

The results of studies are also controversial regarding laboratory findings and their correlation with Ct values. Laboratory findings were also varied among patients grouped based on COVID-19 severity. Elevated interleukin-6 and LDH, as well as decreased lymphocytes and lymphocyte subsets, were reported in deceased patients.[10]. An increase in other inflammatory markers like CRP in patients with severe forms has been highly reported.[25,48,51]

Other factors correlated with COVID-19 severity were decreased lymphocyte and increased neutrophil counts.[25, 51] However, nonconclusive results have been reported for laboratory findings, such as D-dimer.[25,27,51] No significant differences in clinical and laboratory data of saliva from severe COVID-19 patients were observed compared to the critically ill patients in general.[52] CRP, LDH, ferritin, d-dimer, and liver enzyme levels did not significantly differ between critical and noncritical patients.[27] Our data showed significant differences in lymphocyte and neutrophil percentages, LDH, CRP, AST, ESR, and D-dimer among patients based on disease severity. Lymphocyte percentage was lower in severe or critical patients compared to mild or moderate patients. The neutrophil percentage was higher in severe or critical patients than in mild or moderate patients. CRP, LDH, D-dimer, ESR, and AST levels were higher in critical patients.

We also evaluated the utility of Ct values in predicting laboratory and inflammatory factors. In this cohort, we find no significant association between Ct values and laboratory findings except for leukocyte and platelet counts. Contrary to our findings, Jing-Tao Huang et al, found a negative correlation between high viral load (Ct values <30) and the count or percentage of lymphocytes, basophils, eosinophils, $CD4^+$ T cells, and T suppressor $CD8^+$ cells. Furthermore, high viral loads were positively correlated with neutrophil counts and the level of LDH, as well as myocardial enzymes such as CKMB, ultra-troponin I, and N-terminal pro-brain natriuretic peptide.[10] Results of another study revealed a correlation between the Ct values of N1 and N3 regions of the SARS-CoV-2 nucleocapsid and levels of AST, LDH, and ferritin.[52] The mean of absolute lymphocyte counts, as well as D-dimer and CRP levels, differed in patients grouped for Ct values with a cutoff of 34. Patients with Ct values <34 had a lower lymphocyte count and higher CRP levels. However, those patients had lower levels of D-dimer.[46] As mentioned before, different categories of Ct values were used in studies, which might be the reason for the controversial results. Further studies are needed to elucidate the relationship between Ct values and laboratory findings in patients with COVID-19.

The current study may have a number of limitations. There was missing information in several laboratory results. Besides, due to the study period, the study cohort may be comprised of multiple SARS-CoV-2 variants, which may result in the heterogeneity of patients.

In conclusion, the current study retrospectively showed that Ct values could predict the severity of COVID-19. Identifying relevant disease severity and mortality factors is essential to preventing disease progression and saving patients' lives. More studies with larger sample sizes are required to shed light on these controversial findings regarding the correlation between Ct values and laboratory findings.

STATEMENT OF ETHICS

The present study was approved by the Ethics Committee of Tehran University of Medical Sciences (IR.TUMS.IAARI.REC.1399.023). We followed all the principles of ethics in research related to retrospective studies.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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