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Coronavirus Pandemic

Evaluation of the relationship between progression and SARS-CoV-2 viral load in COVID-19 cases in Ankara, Turkey

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Abstract

Introduction: Patients infected with SARS-CoV-2 may present with varying clinical pictures. This study aimed to examine the relationship between viral load cycle threshold value, clinical prognosis and other laboratory parameters in initial swab samples on the day of hospitalization.
Methodology: This retrospective and cross-sectional study included 112 patients, who were diagnosed with SARS-CoV-2 via the Bio-Rad CFX96 Touch™ system. Cycle threshold values for the *RdRp* gene obtained from reverse transcriptase polymerase chain reaction positive patients were recorded.
Results: The mean age of the 112 patients was 47.57 ± 17 years. No relationship was found in symptoms, pneumonia, oxygen need, follow-up in intensive care unit, and mortality between patient groups with cycle threshold values of < 30 and ≥ 30. Frequencies of thrombocytopenia (50%) and elevated LDH levels were higher in patients with cycle threshold values of ≥ 30 ($p = 0.02$ and $p = 0.04$, respectively). There was a weak but significant correlation between cycle threshold values and CRP levels (Pearson's r = 0.207, $p = 0.029$).
Conclusions: Symptoms or clinical prognosis were not significantly related to the SARS-CoV-2 viral load levels tested at admission or for the first time within the scope of this study. Thrombocytopenia and elevated LDH rates were higher in patients with cycle threshold values of ≥ 30. A weak but significant correlation was found between the viral load and CRP levels. Large-scale studies are needed to further elucidate this subject matter.

Key words: SARS-CoV-2, viral load, PCR, prognosis.

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Introduction

Patients infected with the novel coronavirus (2019-nCoV), namely the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), present with varying disease severity ranging from asymptomatic disease to the need for intensive care, and may sometimes lead to mortality [1]. The most common symptoms of coronavirus disease-2019 (COVID-19) include fever, cough, fatigue, myalgia, pain, dyspnea, and diarrhea. Symptom severity in SARS-CoV-2 is associated with mortality rates [2]. Mild clinical symptoms are common in most of the patients with COVID-19, 18-33% of the hospitalized patients need ventilation, and the infection results in mortality in 20% of these patients [3]. Old age, male gender, smoking, and comorbidities including hypertension, cardiovascular disease, and cancer are the risk factors for severe disease and mortality among patients with COVID-19 [4].

Reverse transcription-polymerase chain reaction (RT-PCR) is the standard molecular method used for

the diagnosis of COVID-19 [5]. RT-PCR tests on nasopharyngeal/oropharyngeal (NP/OP) swab samples detect SARS-CoV-2 and provide quantitative information about cycle threshold (Ct) values that are not routinely reported in clinical practice. Ct values are inversely proportional to the viral load [3] and represent the number of amplification cycles required for the target gene to reach a threshold level. Therefore, Ct values can serve as an indirect method to measure the number of viral RNA copies in the sample. However, the sample matrix includes several factors that can affect the efficiency of amplification, and thus affect the Ct values [6]. Viral load measurements in tissue samples are the markers of active virus replication. Such tests are routinely used to follow up clinical progress, response to treatment, recovery, and relapses in severe viral respiratory tract infections [7-9]. The viral load in upper respiratory tract samples of COVID-19 patients was reported to be equally high between asymptomatic and symptomatic patients [10]. The SARS-CoV-2 viral load in COVID-19 patients may be associated with infectivity, disease type, morbidity, and mortality [11-13]. The viral load in NP/OP samples in the SARS-CoV-1 outbreak was associated with deteriorating disease severity and increasing mortality [14,15]. However, the relationship between disease severity and the viral load and viral load dynamics in the lower respiratory tract and other tissue samples in SARS-CoV-2 infections is unknown [13].

In this study we investigated the potential role of the viral load in clinical NP/OP swab tests. Clinical utility of the SARS-CoV-2 viral load Ct values determined in the first NP/OP swab samples on the day of hospitalization was examined to determine whether Ct values were related with selected laboratory parameters and could be used to predict disease severity and the need for oxygen therapy, intensive care, or intubation, and mortality.

Methodology

Study design

This retrospective and cross-sectional study included 112 patients in the age range of 4-99 years. Patients' swab samples were collected on the day of hospitalization in Ankara Gulhane Training and Research Hospital, University of Health Sciences. All the patients were diagnosed with SARS-CoV-2 infection by using the CFX96 Touch™ system (Bio-Rad Laboratories, Hercules, USA).

Ct values of the *RdRp* (RNA-dependent RNA polymerase) gene from the first SARS-CoV-2 RT-PCR tests of patients on the day of hospitalization were recorded. SARS-CoV-2 positive samples with Ct values of < 30 for the *RdRp* gene were called samples with a high viral load and those with Ct values of ≥ 30 were called samples with a low viral load.

The correlation of SARS-CoV-2 viral load Ct values in the first NP/OP swab samples with laboratory parameters and disease severity (pneumonia, oxygen need, intubation, follow-up in the intensive care unit, and mortality) was investigated. Patients' demographic data, clinical prognosis, laboratory parameters, and thoracic computed tomography imaging results were retrieved from the hospital data recording system.

Molecular analysis

Clinical samples collected from patients were transferred into either "Bio-Speedy® COVID-19 Transfer Tubes (BS-NA111)" (VTM, Bioeksen, Istanbul, Turkey) containing viral nucleic acid buffer or transfer tubes containing viral transport media (VTM). The tubes were then transferred to the COVID-19 laboratory while maintaining compliance with the cold chain standards. Extraction was performed after 5-minute incubation in a viral nucleic acid buffer (Bioeksen, Istanbul, Turkey). Samples that were not tested immediately were stored at 4°C.

A sample mix was prepared using a SARS-CoV-2 Bio-Speedy COVID-19 RT-qPCR Detection Kit (Bioeksen, Istanbul, Turkey). The product obtained from the extraction was amplified in a PCR CFX96 Touch™ (Bio-Rad Laboratories, Hercules, USA) or Qiagen Rotor-Gene (QIAGEN, Hilden, Germany) device. The kit enabled rapid diagnosis through a single-step reverse transcription (RT) and real-time quantitative PCR (RT-qPCR) targeting the *RdRp* gene fragment. Positive and negative controls were included in the test according to the manufacturer's protocol. The analytical sensitivity and specificity of the kit were stated by the manufacturer as 99.4% and 99.0%, respectively.

Assessment of the results

The results were assessed in accordance with the manufacturer's instructions. For each target, the device produced a Ct value, which was inversely proportional to the quantitative viral load. The Ct value was not reported to the clinicians. The RT-PCR Ct value represented the value of the first PCR cycle higher than the lowest detection level of the fluorescent signal for the target (i.e., viral RNA). In other words, it represented the number of replication cycles required for sufficient gene amplification to produce fluorescent signals reaching a predefined threshold value.

Ct values were determined in accordance with the manufacturer's instructions. The results for each device were classified as positive or negative. Amplification curves obtained in FAM/HEX channels were analyzed. Non-sigmoidal curves were recorded as negative. The viral load was expressed as the Ct value of SARS-CoV-2's *RdRp* gene. The threshold value was adjusted to 200 RFU for Biorad CFX 96 and 0.02 for Qiagen Rotorgene Q. In the Qiagen Rotorgene Q device, "Dynamic Tube" and "Slope Correct" options were activated and the "Outlier Removal" option was adjusted to 5. If a Ct value was attained by the device for a sample but the curve was not sigmoidal, the result was recorded as negative. Ct values of < 40 and > 40 for SARS-CoV-2 RNA were defined as positive and negative, respectively. When there was not a sigmoidal curve in the negative control but a sigmoid was observed in the sample at a Ct value of > 40, the nucleic acid extract was reanalyzed at -20°C. When another sigmoidal curve was observed again at a Ct value of > 40 in the second analysis, a new sample was obtained from the individual and the analysis was repeated.

Ethical approvals were obtained from the Ethical Committee of the University of Health Sciences, Gulhane Training and Research Hospital (reference number: 2020/12/269).

Statistical Analysis

The SPSS 22 (IBM Corp.) software program was used for statistical analysis. Graphs (histogram and probability plots) and the Kolmogorov-Smirnov test were used to assess whether the variables were normally distributed. The Mann-Whitney U test was used for quantitative variables. Pearson's Chi-Square test or Fisher's exact test was used for qualitative variables. The relationship between SARS-CoV-2's viral load (copy/μL) Ct values and laboratory parameters was assessed by the independent sample t-test and Pearson's correlation coefficient. *p*-values of < 0.05 were accepted as statistically significant.

Results

Of 112 patients included in the study, 57.1% were men. The mean age of the women was 51.58 ± 17.34 years [95% CI: 46.55-56.62] and that of the men was

Table 1. Relationship of cycle threshold (Ct) values with clinical condition.

Characteristics	Cycle threshold (Ct) values			*p* value
	< 30	≥ 30	Total	
Patient	78 (69.6)	34 (30.4)	112	
Gender M/F	44/34	20/14	64/44	
Mean age-years (range)	46.47 ± 18.24	50.10 ± 16.10	47.57 ± 17.62	0.32
Symptoms				
Fever	34 (43.6)	13 (38.2)	47 (42)	0.60
Cough	35 (44.9)	18 (52.9)	53 (47.3)	0.43
Anorexia	1 (1.3)	0	1 (0.9)	1.00
Dyspnea	20 (25.6)	9 (26.5)	29 (25.9)	0.93
Sore throat	9 (11.5)	5 (14.7)	14 (12.5)	0.64
Headache	12 (15.4)	6 (17.6)	18 (16.1)	0.76
Myalgia	6 (7.7)	2 (5.9)	8 (7.1)	0.73
Nausea or vomiting	4 (5.1)	2 (5.9)	6 (5.4)	1.00
Diarrhea	1 (1.3)	4 (11.8)	5 (4.5)	0.03
Anosmia	2 (2.6)	2 (5.9)	4 (3.6)	0.58
Clinical status				
Pneumonia	39 (50)	19 (55.9)	58 (51.8)	0.57
Need for oxygen	15 (19.2)	5 (14.7)	20 (17.9)	0.56
Intubation	7 (9.0)	0 (0)	7 (6.3)	0.09
Needing intensive care	7 (9.0)	0 (0)	2 (1.8)	0.09
Mortality	2 (2.6)	0 (0)	2 (1.8)	1.00
Laboratory values				
Leukocytosis	1 (1.3)	1 (2.9)	2 (1.8)	0.69
Leukopenia	30 (38.5)	12 (35.3)	42 (37.5)	0.69
Lymphopenia	47 (60.3)	19 (55.9)	66 (58.9)	0.67
Anemia	8 (10.3)	7 (20.6)	15 (13.4)	0.14
Thrombocytopenia	21 (26.9)	17 (50)	38 (33.9)	0.02*
AST elevation	11 (14.1)	4 (11.8)	15 (13.4)	0.74
ALT elevation	16 (20.5)	6 (17.6)	22 (19.6)	0.73
LDH elevation	36 (46.2)	23 (67.6)	59 (52.7)	0.04*
CRP elevation	54 (69.2)	26 (76.5)	80 (71.4)	0.44

AST: aspartate aminotransferase; ALT: alanine aminotransferase; LDH: lactate dehydrogenase; CRP: C-reactive protein; *Statistically significant ($p < 0.05$).

44.56 ± 17.36 years [95% CI: 40.23-48.90] (p = 0.71). The most common symptoms on the day of hospitalization were cough (47.3%), fever (42%), and dyspnea (25.9%). Pneumonia was detected in 51.8% (58/112) of all patients, which included 45.3% (29/64) of male patients, and 60.4% (29/48) of female patients. Out of 58 patients with pneumonia, 39 (50%) had a Ct value of < 30 and 19 (32.8%) had a Ct value of ≥ 30 (p = 0.57). The mean ages of patients with pneumonia and without pneumonia were 49.0 ± 17.11 and 46.04 ± 18.19 years, respectively (p = 0.38). Of the patients, 17.9% needed oxygen and 6.3% needed intubation. Only 1.8% of the patients were followed up in the intensive care unit. The infection resulted in mortality in 1.8% of the patients.

Advancing age and viral load Ct values were not correlated (Pearson's r = 0.070, p = 0.463). The rates of oxygen therapy, intubation, intensive care unit stay, and mortality were higher in patients with viral load Ct values of < 30 but the difference was not significant. Leukopenia, lymphopenia, and ALT elevation were found in patients with a high viral load; however, there was not a significant difference. The relationship between the Ct values of 112 patients and the clinical prognosis is presented in Table 1.

The rate of thrombocytopenia (17/34, 50%) was significantly higher in patients with Ct values of ≥ 30 (p = 0.02). Also, the rate of elevated LDH levels was higher in patients with a Ct value of ≥ 30 (p = 0.04). The relationship between viral load Ct values and laboratory findings is presented in Table 2. There was a weak but significant correlation between Ct values and CRP levels (Pearson's r = 0.207, p = 0.029) (Table 3).

Table 3. Correlation between cycle threshold (Ct) values and other parameters using Pearson's correlation coefficient in COVID-19.

Item	Cycle threshold (Ct) values	
	r	*p*-value
Leukocytes	0.095	0.321
Lymphocytes	0.082	0.389
Hemoglobin	-0.162	0.088
Thrombocyte	0.002	0.980
AST	0.056	0.557
ALT	0.032	0.740
LDH	0.083	0.383
C-reactive protein	0.207	0.029*

r: Pearson's correlation. Correlation is significant at the 0.05 level. In COVID-19, there is a significant and weak correlation between Ct values and C-reactive protein. AST: aspartate aminotransferase; ALT: alanine aminotransferase; LDH: lactate dehydrogenase, *Statistically significant (p < 0.05).

Discussion

The analysis of the relationship of Ct values of SARS-CoV-2 viral loads with clinical condition and biochemistry parameters is presented in this study. There are only a few studies in the literature examining the relationship of Ct values on the day of hospital admission or at the time of diagnosis with clinical and laboratory findings. Clinical information about COVID-19 has continuously been accumulating along with published studies; however, there is limited

Table 2. Relationship of cycle threshold (Ct) values with laboratory parameters.

Item	Cycle threshold (Ct) values				*p* value
	Ct < 30		Ct ≥ 30		
	Mean ± SD	95% Cl	Mean ± SD	95% Cl	
Blood routine					
Leukocytes	5.33 ± 2.01 (2.3 - 11.1)	4.88 - 5.79	5.59 ± 2.64 (2.4 - 16.8)	4.67 - 6.51	0.57
Lymphocytes	3.2 ± 12.21 (0.35 - 89)	0.45 - 5.96	3.93 ± 15.04 (0.27 - 89)	0.0 - 9.18	0.79
Hemoglobin	13.93 ± 2.19 (8.9 - 16.5)	13.44 -14.43	13.22 ± 1.79 (9.1 - 16.8)	12.59 - 13.84	0.09
Thrombocyte	203.55 ± 55.65 (28 - 348)	191 - 216.1	204.62 ± 100.03 28 - 534	169.72 - 239.52	0.95
Inflammatory marker					
C-reactive protein	21.09 ± 31.01 (0.4 - 156.6)	14.10 - 28.08	31.91 ± 30.74 (0.0 - 113.2)	0.0 - 312.2	0.09
Blood biochemistry					
AST	33.37 ± 18.67 (14 - 107)	29.16 - 37.58	30.82 ± 29.59 (17 - 76)	26.26 - 35.39	0.47
ALT	32.53 ± 25.99 (9 - 134)	26.67 - 38.39	26.56 ± 14.55 (4-77)	21.48 - 31.64	0.13
LDH	274.41 ± 144.79 (126 - 1103)	241.8 - 307.1	289.88 ± 87.74 (140 - 481)	259.3 - 320.5	0.57

Leukocytes (range: 4.49-10.9×10^3cells/uL); lymphocytes (range: 1.26-3.35×10^3cells/uL); hemoglobin (range: 11.9-14.6 g/dL); thrombocyte (range: 171–388×10^3cells/uL); hemoglobin; AST: aspartate aminotransferase (range: 19-48U/L); ALT: alanine aminotransferase (range: 13-40U/L); LDH: lactate dehydrogenase (range: 0-248 U/L); C-reactive protein (range: 0-5 mg/L).

information on the correlation of viral loads with mortality or prognosis [1].

Although the frequencies of oxygen therapy, intubation, intensive care unit stay, and mortality were higher in patients with a high baseline viral load (Ct < 30) in this study, the figures were not significantly different compared to the group with low viral loads. However, the rate of pneumonia was lower in patients (50%) with high viral load values (Ct < 30) compared to patients (55.9%) with low viral load values. Low Ct values for respiratory samples were associated with more severe disease [16-18]. Mean viral loads in the study by Shi *et al.* were not significantly different across patients with severe pneumonia, and without pneumonia [19]. In another study examining Ct values for NP swab samples, the risk for intubation, ventilator support, or mortality were higher in patients with high SARS-CoV-2 viral loads on the first day of hospitalization [3]. While lower Ct values were associated with more severe disease by some studies [20-22], a lack of correlation was also reported [23].

A study investigating the correlation between SARS-CoV-2 Ct values and mortality was performed in China. That study included 308 hospitalized adult patients and tested Ct values at more than one time point during the disease course and found out that low Ct values indicated the mortality risk. Ct values of non-survivors (median value: 34.79) were reported to be lower than those of patients, who recovered (median value: 37.43) or who continued treatment in the hospital (median value: 36.97) ($p < 0.001$) [24]. In a review of studies comparing viral load and clinical outcomes, it was reported that there was a relationship between SARS-CoV-2 Ct values and clinical outcomes [1]. A variety of SARS-CoV-2 RT-PCR test kits have been developed and approved for use during the pandemic. However, the sensitivity and specificity of such tests may not be standard leading to the suggestion that different Ct values can be reported for the same sample. Moreover, the relationship between Ct values and clinical condition may not be proportionally demonstrated because of the dynamic range of the test and potential inhibitory factors in clinical samples [25].

Ct values have been associated with a range of laboratory markers in different studies. It has been revealed that counts of circulating leukocytes and lymphocytes are normal or decreased in the early stages of COVID-19 disease but can change as the disease progresses [26]. Low Ct values were associated with lower lymphocyte counts in some studies [17,24]. Moreover, it was emphasized that lymphopenia in COVID-19 patients might be a marker of high disease severity [27,28].

When the relationship between viral load (Ct) values and some laboratory parameters was assessed, a weak but significant correlation was found between Ct values and CRP levels. No correlations were found between Ct values and other laboratory parameters obtained on the first day of hospitalization. As a biochemical parameter, LDH is known to indicate tissue destruction and it is an important prognostic marker for lung injury. Lower Ct values were associated with high LDH levels [17,18,24]. It was reported in some studies investigating the relationship between LDH levels and Ct values that elevated LDH levels could be a marker of poor prognosis in COVID-19 patients [28,29]. Contrary to earlier reports, elevated LDH levels were more commonly observed in patients with Ct values of ≥ 30 in our study. Thrombocytopenia is another finding shown to be a risk factor for leukopenia, lymphopenia, and elevated CRP and LDH levels in patients with severe COVID-19 [30,31]. In this study, thrombocytopenia was detected in patients and it was more common in patients with Ct values of ≥ 30 ($p = 0.02$). These results suggest that LDH elevation and thrombocytopenia can occur independently of the viral load and that such patients require closer follow-up. Reporting SARS-CoV-2's viral load and Ct values for NP swab samples to clinicians is considered a useful strategy to follow high-risk individuals closely [16,32].

Regardless of the asymptomatic or symptomatic course of COVID-19, detection of SARS-CoV-2 by RT-PCR and information about the viral load can contribute to the clinical assessment of the patient at any stage of the infection [33]. However, Dahdouh *et al.* [34] reported the lack of standardization in determining the SARS-CoV-2 viral load in clinical samples. Variations may occur in the Ct values obtained from the same sample because NP swab sample contents for the PCR test may vary due to operational differences and patient tolerance during the collection of the sample. Furthermore, extraction and amplification methods may vary resulting in variable Ct values. For these reasons, it has been reported that the Ct value may be misleading because it may not reflect the actual viral load in the patient's nasopharynx.

Conclusions

In this study, a significant relationship was not found between clinical prognosis and SARS-CoV-2 viral load levels at the time of hospital admission or on the day of the first PCR test. The rates of thrombocytopenia and elevated LDH levels were higher

in patients with Ct values of ≥ 30. A weak but significant correlation was found between the viral load and CRP levels. Duration of the disease, sample types, and RT-PCR tests can affect the quantification of the viral load. Further studies on larger populations are needed.

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