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Research Article

Association of Initial Viral Load of SARS-CoV-2 with Clinical Progression and Mortality in Hospitalized Patients with COVID-19

Sema Alacam,[1] Ayfer Bakir,[2] Nagehan Didem Sari[3]

[1]Department of Medical Microbiology, University of Health Sciences, Istanbul Training and Research Hospital, Istanbul, Turkey
[2]Department of Medical Microbiology, University of Health Sciences, Gulhane Training and Research Hospital, Ankara, Turkey
[3]Department of Infectious Diseases and Clinical Microbiology, University of Health Sciences, Istanbul Training and Research Hospital, Istanbul, Turkey

Abstract

Objectives: The relationship between the clinical course of COVID-19 and viral load has not been fully elucidated, and conflicting results are still reported. In this study, we aimed to investigate the relationship of initial viral load with clinical progression and mortality in hospitalized cases.

Methods: This study consisted of 218 patients with moderate and severe disease. SARS-CoV-2 cycle threshold (Ct) values and positivity were determined in the nasopharyngeal swab samples of the patients by real-time PCR.

Results: The mortality rate in hospitalized COVID-19 patients was 15.6%. A weak but significant correlation was found between increasing age and mortality (Spearman's rho: 0.181, p=0.007). The first detected SARS-CoV-2 viral load (Ct value) of the patients who were admitted to the clinical and intensive care unit were 29.7 and 27.9, respectively (p=0.07). Initial median viral loads of patients who recovered and died were 29.4 and 28.9, respectively (p=0.44).

Conclusion: Initial viral load could not be associated with the severity of the disease and the risk of mortality in moderate and severe COVID-19 patients. This study suggests that viral load is not a reliable parameter in predicting COVID-19 prognosis and mortality.

Keywords: Ct value, mortality, real time-PCR, SARS-CoV-2, viral load

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Severe acute respiratory syndrome (SARS-COV-2), which spread rapidly in the world and caused the death of millions of people, caused 5,319,359 confirmed cases of Coronavirus disease 2019 (COVID-19) in Turkey as of June 11, 2021, and 48,593 of them died.[1] SARS-CoV-2, the causative agent of COVID-19, is a new highly contagious human pathogen belonging to the *Betacoronavirus* genus of the *Coronavidae* family. In the last two decades, it is the third major agent to cause a pandemic in 2019, after SARS-CoV in 2002 and Middle East respiratory syndrome (MERS) in 2012.[2,3] While most patients with COVID-19 are clinically asymptomatic or with mild symptoms,[4] 18% to 33% of hospitalized patients require mechanical ventilation support.[5,6] Data from meta-analyses support the association of advanced age (≥65 years), male gender, and comorbidities such as diabetes mellitus, hypertension, cardiovascular diseases, chronic obstructive pulmonary disease and cancer with a high mortality rate in patients with COVID-19 pneumonia.[7]

The pathogenesis of COVID-19 is still not fully understood. Cytokine storm is thought to play an important role in dis-

Address for correspondence: Sema Alacam, MD. Saglik Bilimleri Universitesi Istanbul Egitim ve Arastirma Hastanesi Tibbi Mikrobiyoloji Anabilim Dali, Istanbul, Turkey
Phone: +90 532 682 20 17 **E-mail:** semalacam@hotmail.com

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ease severity.[8] Some studies have focused on the initial viral load in respiratory samples as a marker of severe COVID-19 disease.[9] It has been suggested that the viral load determined by the cycle treshold (Ct) value will help in determining the clinical course, intubation and mortality risk in hospitalized patients and should be reported to clinicians.[10] Therefore, viral load can be used as a useful marker to predict severe disease, as well as to determine the need for aggressive treatment and intensive care.[11]

The relationship between the clinical course of COVID-19 and viral load has not been fully elucidated, and conflicting results are still reported. Therefore, in this study, we aimed to investigate the relationship of initial viral load with clinical progression and mortality in hospitalized cases.

Methods

Study Design

This study includes 218 COVID-19 patients, aged between 18-93, with moderate to severe disease manifestation between 2 March 2020 – 1 September 2020. The clinical symptoms, laboratory, and radiological data of the patients were obtained from the patient files from the hospital information management system.

Patients admitted to the emergency department were divided into three groups according to their symptoms as mild, moderate and severe disease. Patients with positive SARS-CoV-2 PCR test, hospitalized and moderate to severe disease were included in the study. Distinctive features of moderate disease; the presence of clinical or radiological evidence of lower respiratory tract disease. In addition, the patient's blood oxygen saturation (SpO_2) should be ≥94% while the patient is breathing ambient air. Indicators of severe disease; tachypnea (respiratory rate ≥30 per minute), hypoxemia (SpO_2 <94%), and radiological evidence of lower respiratory tract disease.

Molecular Analysis

Samples collected with synthetic fiber swabs were inserted into a sterile vNAT transfer tube containing 2 ml extractive and preservative vNAT (viral nucleic acid buffer) (Biospeedy, Bioeksen, Istanbul, Turkey). The collected samples were stored at +2-8°C and transferred to the Virology COVID-19 Laboratory of our hospital under the same conditions. Since the liquid in the vNAT tubes provided the extraction of SARS-COV-2 RNA in 5 minutes, the PCR step was started directly without the need for intermediate processing. The diagnosis with the Biospeedy SARS-COV-2 Double Gene RT qPCR Version 4 (Bio-speedy, Bioeksen, Istanbul, Turkey) kit was performed with one-step real-time reverse transcription (RT) PCR targeting the SARS-CoV-2-specific N gene and Orf1ab gene region.

The PCR reaction mix was prepared to contain 5 µL of Oligo Mix, 10 µL of 2X Prime Script Mix and 5 µL of RNA for each sample. Real-time RT-PCR was performed on the Bio-Rad CFX96 Touch Instrument (Bio-Rad, USA).

PCR tests were evaluated in accordance with the recommendations of the kit manufacturer. Sigmoid curves with a Ct value less than 38 were defined as positive for SARS-CoV-2. Ct is defined as the number of cycles required for the fluorescent signal to cross the threshold (exceed the background level). Ct levels are inversely proportional to the amount of target nucleic acid in the sample. Thus, the lower the Ct level determined for a sample, the higher the viral load in that sample.[12] We used Ct values, which semi-quantitatively determine the level of viral load in the sample in our study.

Statistical Analysis

SPSS 22 (IBM Corp) program was used for statistical analysis. The conformity of the variables to the normal distribution was evaluated with visual methods (histogram and probability graphs) and Kolmogorov-Smirnov test. Mann-Whitney U test and Kruskal Wallis were used for quantitative variables, Pearson Chi-Square or Fisher exact tests were used for qualitative variables. The correlation between increasing age and mortality and between comorbidity and mortality was evaluated with Spearman's analysis test. Results with a P value below 0.05 were considered statistically significant.

Results

A total of 218 cases, of which 156 (71.6%) had moderate disease and were admitted to the clinical service, and 62 (28.4%) had severe disease and were admitted to the intensive care unit, were included in this study. The median age of all cases was 60, and the median age of those with severe disease was 66 [interquartile range (IQR): 59-76] (p<0.001). In general, antiviral was used in 127 (58.3%) patients, antibiotics in 179 (82.1%) and hydroxychloroquine in all (100%) patients. Demographic, clinical and therapeutic characteristics of COVID-19 patients are presented in Table 1. The COVID-19 death rate in hospitalized patients was found to be 15.6%. This rate was 12% (13/108) in women and 19.1% (21/110) in men, and this difference was not statistically significant (p=0.15). There was a weak but significant correlation between mortality and advanced age (Spearman's rho: 0.181, p=0.007). The first detected SARS-CoV-2 viral load (Ct value) of the patients who were admitted to the clinical and intensive care unit were 29.7 (IQR: 26.5-32.5) and 27.9 (IQR: 25.5-31.2) respectively (p=0.07). Initial viral load of patients who recovered and died were 29.4 (IQR: 26.4-32.4) and 28.9 (IQR: 25.5-31.4), respectively (p=0.44) (Fig. 1).

Table 1. Demographic and clinical characteristics of patients with SARS-CoV-2 infection by severity of disease

Characteristics	Total (n=218)	Moderate (n=156)	Severe (n=62)	p
Age (median; IQR)	60 (51-69)	58 (50-68)	66 (59-76)	<0.001
Gender (M/F)	1.02/1	1.12/1	0.8/1	
Male	110 (50.5)	82 (52.6)	28 (45.2)	0.32
Female	108 (49.5)	74 (47.4)	34 (54.8)	
Ct (median, IQR)	29.3 (26.3-32.3)	29.7 (26.4-32.5)	27.9 (25.4-31.2)	0.07
Mortality	34 (15.6)	18 (11.5)	16 (25.8)	0.009
Comorbidity (Total)	159 (72.9)	97 (62.2)	62 (100)	<0.001
Diabetes mellitus	85 (39)	51 (32.7)	34 (54.8)	0.002
Hypertension	102 (46.8)	59 (37.8)	43 (69.4)	<0.001
Heart disease	35 (16.1)	14 (9)	21 (33.9)	<0.001
Lung disease	33 (15.1)	13 (8.3)	20 (32.3)	<0.001
Renal disease	14 (6.4)	4 (2.6)	10 (16.1)	<0.001
Malignancy	22 (10.1)	8 (5.1)	14 (22.6)	<0.001
Symptoms				
Fever	125 (57.3)	89 (57.1)	36 (58.1)	0.89
Cough	129 (59.2)	89 (57.1)	40 (64.5)	0.31
Shortness of breath	56 (25.7)	36 (23.1)	20 (32.3)	0.16
Weakness	129 (59.2)	94 (60.3)	35 (56.5)	0.61
Nausea	32 (14.7)	21 (13.5)	11 (17.7)	0.42
Diarrhoea	12 (5.5)	9 (5.8)	3 (4.8)	0.79
Sore throat	23 (10.6)	18 (11.5)	5 (8.1)	0.45
Myalgia	34 (15.6)	24 (15.4)	10 (16.1)	0.89
Anorexia	41 (18.8)	30 (19.2)	11 (17.7)	0.80
Chest CT				
Unilateral involvement	18 (8.3)	14 (9)	4 (6.5)	0.78
Bilateral involvement	184 (84.4)	130 (83.3)	54 (87.1)	
No involvement	16 (7.3)	12 (7.7)	4 (6.5)	
Treatment				
Antiviral	127 (58.3)	86 (55.1)	41 (66.1)	0.14
Antibiotics	179 (82.1)	128 (82.1)	51 (82.3)	0.97
Hydroxychloroquine	218 (100)	156 (100)	62 (100)	

* Data are n (%); age: years, median (interquartile range); Ct: cycle treshold; IQR: Interquertile range; ICU: intensive care unit admission; statistically significant p values are in bold.

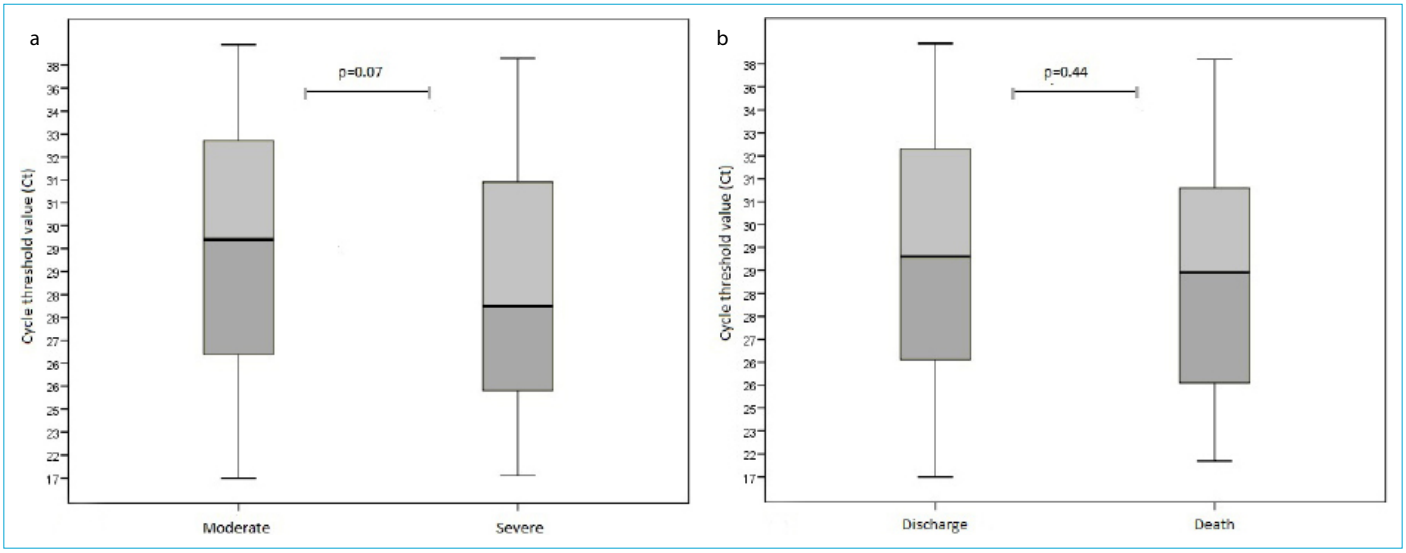

Figure 1. Box-plot graph of SARS-CoV-2 viral load cycle threshold (Ct) values of hospitalized 218 COVID-19 cases **(a)** cases with moderate to severe disease **(b)** discharged and death cases.

Lymphocyte levels of patients with severe disease were found to be lower than those with moderate disease (p=0.03). Lactate dehydrogenase (LDH) and D-dimer levels were above reference values in patients with both moderate and severe disease. In addition, C-reactive protein (CRP) levels were significantly higher in patients with severe disease compared to others (p=0.002). In our study, hematological, biochemical, coagulation and inflammatory parameters of moderate and severe COVID-19 patients are shown in Table 2 comparatively.

Comorbidity was detected in 62.2% (97/156) of those with moderate disease and in all of those with severe disease. The most common comorbidities in all patients were hypertension (46.8%) and diabetes mellitus (39%). Most of the patients developed fatigue (59.2%), cough (59.2%) and fever (57.3%). There was evidence of bilateral involvement on chest computed tomography in 184 (84.4%) of the patients. When the relationship between comorbid diseases and mortality in patients with COVID-19 was evaluated, a weak but significant correlation was found (Spearman's rho: 0.150, p=0.03) (Table 3). Comorbid disease was present in 27 (79.4%) of 34 patients who died. Eight of them had at least one comorbid disease and 19 had more than one comorbid disease (p=0.44). Comorbid diseases in patients who died in our study were diabetes mellitus (17, 50%), hypertension (17, 50%), malignancy (7, 20.6%), chronic obstructive pulmonary disease (6, 17.6%), ischemic heart disease (4, 11.8%), chronic renal failure (4, 11.8%). The initial viral loads of the subjects who died were 28.7 (IQR: 26.6-33.2) in those with comorbid disease and 30 (IQR: 29.5-30.9) in those without (p=0.69).

Table 3. Relationship between comorbid diseases and mortality in COVID-19 cases

	Spearman's rho (r)	p
Diabetes mellitus	0.097	0.15
Hypertension	0.028	0.69
Chronic renal failure	0.042	0.54
Ischemic heart disease	-0.050	0.46
Chronic obstructive pulmonary disease	0.030	0.66
Malignancy	0.150	0.03

Discussion

In this retrospective cross-sectional study, we investigated the effect of viral load on the clinical characteristics (moderate and severe disease) and prognosis of COVID-19 patients. PCR-detected initial viral loads of surviving and deceased patients on the day of admission to the hospital were evaluated, and no significant difference could be demonstrated. In addition, no significant difference was found between baseline viral loads of cases with moderate and severe disease symptoms. In some previous studies, it was emphasized that viral load is important in the prognosis of the disease.[9,10,13] Magleby et al.[10] argued that the risk of death is associated with high viral load. Liu et al.[13] reported that the viral load was higher in severe cases of COVID-19 than in mild cases. Fajnzylber et al.[14] also report-

Table 2. Comparison of laboratory parameters between patients with moderate and severe COVID-19

Characteristics	Moderate	Severe	p
Hematological parameters			
WBC (4.23-10.2 x10^3 cell/uL)	6 (4.7-7.7)	6.4 (4.5-8.7)	0.50
Neutrophil (1.56-6.13 x10^3 cell/uL)	3.6 (2.7-5.2)	4.5 (2.7-6.4)	0.14
Lymphocyte (1.18-3.57 x10^3 cell/uL)	1.3 (0.8-1.9)	1.1 (0.7-1.6)	0.03
Hemoglobin (12.2-16.2 g/dL)	13 (12-14)	12.8 (9-14)	0.20
Platelet (142-424 x10^3 cell/uL)	210 (165-255)	218 (167-274)	0.48
Biochemical parameters (References range)			
ALT (<33 U/L)	22 (15-31)	21 (13-30)	0.31
AST (<32 U/L)	28.5 (23-38)	30 (23-41)	0.74
LDH (135-214 U/L)	234 (179-312)	221 (168-352)	0.92
Creatinin kinase (26-192 mg/dL)	62 (35-128)	65 (30-91)	0.43
Urea (12.84-42.8 mg/dL)	29 (22-41)	35 (24-62)	0.04
Creatinine (0.5-0.9 mg/dL)	0.9 (0.69-1)	1 (0.71-1.7)	0.34
Coagulation parameters			
D-dimer (<0.55 ng/mL)	0.6 (0.28-1.12)	0.8 (0.35-2)	0.13
Inflammatory parameters			
C- reactive protein (<5 mg/L)	19.9 (6.1-72.9)	51.1 (14.2-131)	0.002

*Data are median (IQR); WBC: white blood cells; ALT: alanine aminotransferase; AST: aspartate aminotransferase; LDH: lactate dehydrogenase.

ed that viral load plays a role in the severity and mortality of COVID-19. However, in one cohort study, the researchers showed no significant difference between viral load and clinical course. The different results in the studies may be due to the fact that the sampling time was at different stages (coincidence) of the disease.[15] On the other hand, viral load rates may be affected because SARS-CoV-2 PCR testing is performed with nasopharyngeal swab samples from a mucosal surface with variability. In addition, the use of different extraction and amplification methods in the PCR test may cause differences in viral load values.[16] Currently, age, gender and comorbidity are reported as high risk factors for COVID-19 patients in epidemiological studies.[17] It has been reported that SARS-CoV-2 infection is more likely to affect especially elderly men with comorbidities depending on gender and may lead to fatal respiratory diseases such as acute respiratory disease syndrome.[4,7] Interestingly, SARS and MERS also infected more males.[8,18] In men and women, levels of sex hormones differ significantly in neurocognitive aging process, immune function, vascular health, response to therapeutics. The impact of these factors on the relationship between gender and prognosis has been one of the main topics discussed in recent reviews.[19-21] It has also been shown in previous studies that sex hormones are effective in regulating innate and adaptive immune responses.[22] Including confirmed cases of COVID-19 in many countries, it shows that men requiring hospitalization are 50% more common than women and three to four times higher admission to intensive care unit.[23, 24] A meta-analysis study examining the probability of developing COVID-19 infection by gender found that this ratio was 1.31 in men compared to women.[25] Similar to other studies, the majority of patients hospitalized for moderate to severe disease in this study were male patients, and mortality was higher in males. However, no statistically significant difference was found. Increased age is a well-known strong risk factor for COVID-19 mortality.[26] Our study also confirms the high mortality rate due to severe COVID-19 among elderly patients.

Comorbidities are the leading cause of conversion to severe and critical cases in patients with COVID-19. The risk factors for COVID-19 mortality in patients with different comorbidities are not the same.[27] The risk of death from COVID-19 is largely dependent on previous health conditions and age. Elderly patients and those with chronic comorbidities such as cardiovascular disease, hypertension, diabetes mellitus, and lung disease are much more prone to critical and fatal disease outcomes.[4,28] Detailed information on the contribution of comorbidity to death in COVID-19 patients who have died is still lacking. Understanding this mechanism is only possible with autopsy.[29] In our study, comorbidities were significantly higher in the group with severe disease, and most of the patients who died (79.4%) had comorbidities. Since these patients do not have autopsy information, the causality and mechanism of death are not known precisely.

In this study, the initial biochemical, hematological, coagulation and inflammatory parameters of moderate and severe COVID-19 patients were examined. CRP, LDH and D-dimer values were found to be higher than the reference range only in moderate and severe patients. Changes in lymphocyte, CRP, LDH and D-dimer values can be important supportive parameters in predicting poor prognosis and may guide early identification of critical cases and treatment decisions.[30]

Limitation of the study; as the study was retrospective, information on the day the first nasopharyngeal swab sample was obtained from the onset of symptoms was not available.

Conclusion

In conclusion, baseline viral load values could not be associated with disease severity and mortality risk in moderate to severe COVID-19 patients in this study. Therefore, considering the results of this study, it is suggested that viral load is not a reliable parameter in predicting COVID-19 prognosis and mortality.

Disclosures

Ethics Committee Approval: The study protocol was approved by Istanbul Education Research Hospital Clinical Research Ethics Committee with 19/02/2021 and 2733 number and Helsinki Declaration decision.

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

Authorship Contributions: Concept – S.A., A.B.; Design – S.A., A.B.; Supervision – S.A., A.B., N.D.S.; Materials – S.A., N.D.S.; Data collection &/or processing – S.A., A.B., N.D.S.; Analysis and/or interpretation – S.A., A.B.; Literature search – S.A., A.B.; Writing – S.A.; Critical review – S.A., A.B., N.D.S.

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