Relationship between RT-PCR Threshold Cycle Values and Thorax CT Severity Score in COVID-19 Patients

COVID-19 Hastalarında RT-PCR CT Değerleri ile Toraks BT Şiddet Skoru Arasındaki İlişki

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ÖZ

Amaç: Bu çalışmada, viral yük hakkında bilgi veren RT-PCR Cycles Threshold (Ct) değerleri ile akciğer tutulumunu göstermek için kullanılan Bilgisayarlı Tomografi şiddet skoru (BTŞS) arasındaki ilişkinin araştırması amaçlandı.

Araçlar ve Yöntem: 1 Ocak 2021 ile 31 Kasım 2021 tarihleri arasında akciğer oskültasyon bulguları ve/veya posteroanterior akciğer grafisinde şüpheli bulguları olan ve RT-PCR testi pozitif çıkan toplam 162 hasta çalışmaya dahil edilmiştir. Olguların viral yükleri, akciğer tutulumu ve mortalite oranları hastaneye yatırıldıkları birimlere göre analiz edildi. Ayrıca, COVID-19, BTŞS, RT-PCR Ct viral yük değerleri ve yaşa eşlik eden komorbid hastalıklar arasındaki korelasyon incelendi.

Bulgular: BTŞS yaş ile pozitif ve anlamlı bir korelasyon göstermiştir (p=0.017). İstatistiksel analiz, viral yük ile torasik tutulum arasında anlamlı bir korelasyon olmadığını ortaya koymuştur (p=0.663).

Sonuç: Bu çalışmaya göre, COVID-19 hastalığının tanısında önemli bir yer tutan RT-PCR Ct değerleri ile toraks BT tutulumu arasında anlamlı bir korelasyon bulunmazken, akciğer tutulumu prevalansı ve olguların ölüm oranı yaşla birlikte artmıştır. Ayrıca, CT-CS skoru ile yaş arasında anlamlı bir korelasyon gözlenmiştir. Bu bulgular, bu alanda gelecekte yapılacak derinlemesine araştırmaların önünü açarak konunun daha iyi anlaşılmasını sağlayacaktır.

Anahtar Kelimeler: bilgisayarlı tomografi; COVID-19; tomografi; torasik tutulum; viral yük

ABSTRACT

Purpose: In this study, we aimed to investigate the relationship between RT-PCR Cycles Threshold (Ct) values, which provide information about the viral load, and CT severity score (CTSS), which is used to demonstrate lung involvement.

Materials and Methods: Between January 1, 2021 and November 30, 2021, a total of 162 patients with lung auscultation findings and/or suspicious findings on posteroanterior chest radiography, along with a positive RT-PCR test, were included in the study. Viral loads, lung involvement, and mortality rates of the cases were analyzed based on the units in which they were hospitalized. Additionally, we investigated the correlation between comorbid diseases associated with COVID-19, CTSS (CT severity score), RT-PCR Ct viral load values, and age.

Results: The CTSS showed a positive and significant correlation with age (p=0.017). Statistical analysis revealed no significant correlation between viral load and thoracic involvement (p=0.663).

Conclusion: According to the present study, no significant correlation was found between RT-PCR Ct values, which play a crucial role in the diagnosis of COVID-19, and thoracic CT involvement. However, the prevalence of lung involvement and the mortality rate of the cases increased with age. Additionally, a significant correlation was observed between the CT severity score (CT-SS) and age. These findings pave the way for future in-depth research in this field, fostering a better understanding of the subject matter.

Keywords: computed tomography; COVID-19, thoracic involvement; tomography; viral load

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INTRODUCTION

SARS CoV-2 (COVID-19), the causative agent of coronavirus disease, is transmitted through the respiratory tract and close contact.[1] The real-time reverse transcriptase polymerase chain reaction (RT-PCR), used in diagnostic purposes, provides a quantification of viral load in the sample through the cycle threshold (Ct) value.[2] The Ct value is an important determinant in assessing the likelihood and severity of disease transmission.[3,4] Lung involvement is the main cause of morbidity and mortality in COVID-19 patients.[2] Due to its wide availability and rapid examination time, the role of chest computed tomography (CT) is increasingly complementary to RT-PCR in patients with COVID-19 pneumonia.[5] Chest CT examination holds significance not only for the prompt diagnosis of COVID-19 but also for disease monitoring and assessing the effectiveness of treatment.[6,7] The utilization of Computed Tomography Severity Scoring (CTSS) is recommended for quantifying pulmonary involvement in patients with COVID-19 and establishing correlations with clinical classifications.[6] The objective of this study was to examine the correlation between RT-PCR Ct values, serving as viral load markers, and CTSS values among hospitalized patients with COVID-19.

MATERIALS and METHODS

This study was conducted with the approval of the Ministry of Health, General Directorate of Health Services Scientific Study Platform (Decision No: T21-31-28, Date: 11-10-2021) Approval for this study was obtained from Aksaray University Clinical Research Ethics Committee (dated 16.12.2021 and numbered 2021/17-03).

Collection of Samples

The study included patients aged 18 and above, with confirmed positive RT-PCR tests, and without any known pre-existing lung disease, who were admitted to the COVID-19 service and intensive care unit during the period of January 1, 2021, to November 30, 2021. In the study, the cases were analyzed in terms of demographic information (age, gender), comorbid diseases (diabetes, hypertension, cardiovascular diseases, etc.), CTSS, RT-PCR Ct values, the unit of hospitalization (ward-intensive care unit) and the outcome of the disease (non-survivor/survivor).

Molecular Studies and RT-PCR

Naso-oropharyngeal swab samples were collected with a COVID-19 transfer tube (vNAT, Bioeksen, Istanbul, Turkey) and sent to the laboratory. Nucleic acid extraction and PCR reaction was performed without modification according to the manufacturers' instructions contained in the kit inserts (Bio-Speedy COVID-19 RT-qPCR Detection Kit) (Bioeksen, Turkey). Amplification was performed on a Bio-Rad CFX96 Touch™ (Bio-Rad, USA). RT-PCR Ct values <25 were determined as high, 25-30 as moderate and >30 as low viral load.[8]

Determination of Lung Involvement and Severity Scores by CT

Multidetector CT device (GE Medical Systems, USA) was used for CT scanning. CT scanning was performed according to the manufacturer's recommendations. Images were reconstructed to include coronal and sagittal planes with 1-mm slice thickness. In the determination of computed tomography total severity score (CT-CS), a semi-quantitative scoring system was used to determine the lung involvement of COVID19-related abnormalities.[6] Each lung lobe was scored from 0 to 5 (0: No lobe involvement; 1: <5% of the lobe; 2: 5-25% of the lobe; 3: 26-50% of the lobe; 4: 51-75% of the lobe; 5: >75% of the lobe involved). Along with this scoring; total severity scoring was performed on CT. Values of 8 and below were accepted as mild, values between 9 and 15 as moderate and values above 15 as severe lung involvement.[9] In addition, the patients were grouped as Group I without lung involvement, Group II with mild involvement (Figure I), Group III with moderate involvement (Figure II)and Group IV with severe involvement (Figure III).[10]

Statistical Analysis

IBM Statistics for Windows, Version 26.0 package program was used to evaluate the data. Parametric and/or nonparametric test methods were used for comparison of continuous variables between groups according to Shapiro Wilk normality test. Student's t test or Mann

Whitney U test were used to compare two independent groups, and One-Way ANOVA and Kruskal Wallis tests were used to compare more than two independent groups. If the difference was found to be significant, the groups that caused the difference were identified by using appropriate posthoc multiple comparison tests. The difference was defined with letter indices placed on the means or medians. As descriptive statistics, mean±St.deviation median (minimum-maximum) was used to summarize continuous variables, while frequency distributions and percentages were used for categorical variables. Multiple Logistic Regression method (Backward LR option) was used to evaluate the risk factors affecting mortality and the results are presented with odds ratios and confidence intervals.

Figure I. Mild Lung Involvement CT Image.

Figure II. Moderate Lung Involvement CT Image.

Figure III. Severe Lung Involvement CT Image.

RESULTS

A total of 162 patients, 77 females and 85 males, who were positive for COVID-19 RT-PCR, aged 18 years or older, had lung listening findings on physical examination and/or suspicious findings on posteroanterior chest radiography were included in the study. In addition, the cases were divided into 3 groups as low (Ct>30), intermediate (Ct 25-30) and high (Ct<25) viral load according to RT-PCR Ct value. The mean age of the patients was 63.42 years, 133 were hospitalized in the ward and 29 in the intensive care unit (Table 1).

Lung involvement rates and RT-PCR Ct values of cases were compared. No association was observed between lung involvement and viral load, although it was observed that mortality increased as lung involvement increased (Table 2).

It was determined that COVID-19 patients had comorbid diseases (diabetes mellitus, hypertension, cardiovascular diseases, malignancy, rheumatologic diseases, renal diseases, obesity, neurologic diseases and endocrine disorders). The most common comorbid diseases were hypertension (n=63, 38.88%), cardiovascular disease (n=44, 27.16%), diabetes mellitus (n=36, 22.22%) and chronic obstructive pulmonary disease (n=11, 6.79%). The correlation between these diseases, CTSS and RT-PCR Ct viral load values and age were analyzed. A significant positive correlation was found between CTSS score and age (r=0.188, p=0.017) (Table 3).

We compared the variables of Ct viral load, age, and number of chronic diseases with CT severity categories, and found that the difference was significant only for age.

The mean age of the group with mild viral load was statistically lower than the group with moderate and severe viral load (Table 4).

Table 1. Viral loads of the cases.

Parameters	Viral loads	High <25 n (%)	Mild 25-30 n (%)	Low >30 n (%)	Total n (%)
ICU	Man	6	5	7	18
	Woman	3	6	2	11
Ward	Man	29	20	18	67
	Woman	27	20	19	66
Total	Man	35	25	25	85 (52.47)
	Woman	30	26	21	77 (47.53)
	Total	65 (40.12)	51 (31.48)	46 (28.40)	162 (100)

ICU: Intensive Care Unit

Table 2. CT Lung involvement, viral loads and non-survivor rates.

Groups	Lung Involvement	Viral Load (Ct)	Non-survivor rates n (%)	Survivor rates n (%)	Total n (%)
I	No involvement	20-32	0 (0)	2 (100)	2 (100)
II	Mild	16-39	2 (4)	48 (96)	50 (100)
III	Moderate	12-38	11(18.97)	47 (81.03)	58 (100)
IV	Severe	17-39	16 (30.77)	36 (69.23)	52 (100)
Total			29 (17.9)	133 (82.1)	162 (100)

Table 3. Correlation between CTSS, Ct viral load, age and comorbid diseases.

Parameters		CTSS	Ct Viral Load	Age	Number of Comorbid Disease
	r		0.035	**0.188**	-0.001
CTSS	p		0.663	**0.017**	0.990
	n		162	162	124
	r	0.035		-0.021	-0.059
Ct Viral Load	p	0.663		0.792	0.513
	n	162		162	124
	r	**0.188**	-0.021		0.135
Age	p	**0.017**	0.792		0.136
	n	162	162		124
Number of Comorbid Disease	r	-0.001	-0.059	0.135	
	p	0.990	0.513	0.136	
	n	124	124	124	

CTSS: Computed Tomography Severity Scoring, r:Pearson's correlation coefficient (rho),p:p value,n:number of cases

Table 4. Comparison of CT viral load, age and number of chronic diseases variables and CT severity score categories.

CT Severity Categories	Mild	Moderate	Severe	p
Ct Viral Load	26.9±6.07 27(16-38)	26.69±6 27(12-39)	26.86±5.39 26(17-39)	0.977
CTSS	4.58±2.07 5(1-8)[a]	12.15±2.83 12(8-17)[b]	20.74±1.83 21(18-25)[c]	<0.001
Age	55.78±20.01[a] 60(18-87)	65.76±14.1[b] 67(18-90)	66.94±12.93[b] 66(38-88)	0.001
Number of Chronic Diseases Variables	1.85±0.73 2(1-3)	1.75±0.85 2(1-4)	1.77±1.01 1.5(1-5)	0.969*

CTSS: Computed Tomography Severity Mean±St.Dev. and Median (Min.-Max.),*; p value from Kruskal Wallis test and all others from ANOVA
[a,b,c]; indices such as a,b and c shows statistically difference of means or medians.

DISCUSSION

In this study, we investigated the relationship between RT-PCR Cycles Threshold (Ct) values, which provide information about the viral load carried by the individual, and CT-CS, used to demonstrate lung involvement. Our findings revealed that no significant correlation was found between RT-PCR Ct values, which play an essential place in the diagnosis of COVID-19 disease, and thoracic CT involvement, whereas the prevalence of pulmonary involvement and the mortality rate of cases increased with age.

The detection of COVID-19 relies on the reference method of reverse transcription polymerase chain reaction (RT-PCR), which allows for the identification of specific viral RNA sequences.[7] The Ct value obtained from this test is inversely correlated with the viral load carried by the patient.[11,12] In a study by Calle et al. involving 455 patients, a higher respiratory risk was associated with higher viral load, as indicated by lower Ct values.[13] Simi-

larly, Singh et al. found that symptomatic COVID-19 patients had significantly lower RT-PCR Ct values compared to those without clinical symptoms.[12] In another study conducted by Shah et al. in India with 219 patients, lower Ct values were found to be indicative of increased disease severity.[14] In a study conducted in Mersin, it was observed that symptomatic patients had a higher mean age compared to asymptomatic patients, and their Ct values were lower.[5] Similarly, Bakir et al. conducted a study in Ankara involving 158 patients, and like our study, they reported no association between Ct values and age, gender, or mortality.[15] These findings collectively highlight the importance of Ct values in assessing viral load and the clinical implications of such measurements.

Our study did not find any significant relationship between RT-PCR Ct values and lung involvement.

In cases with COVID-19 pneumonia, imaging using CT is important in the detection and evaluation of lung infection.[16,17] In COVID-19 pneumonia, CT findings are similar to those seen in other viral pneumonias. These CT manifestations predominantly present as areas of ground-glass opacities and consolidations.[7,18] The CTSS value is used to rapidly and objectively assess the severity of pulmonary involvement in COVID-19 patients.[7] In a study conducted by Yağcı et al. including 284 patients, although the CTSS value was found to be higher in hospitalized patients, an inverse relationship was found between viral load and thoracic severity score, and it was found that viral load was not an effective factor in hospitalization and mortality.[19] Similar to this study, we did not find a significant correlation between CTSS value and viral load. We observed that all deceased patients were in intensive care units, and the probability of mortality increased with advancing age. Francone et al. found that the mortality risk was higher in patients with a CTSS of 18 or higher and Li et al. found that the mortality risk was higher in patients with a CTSS of 15 or higher.[20,21] According to Saeed et al, a severe clinical picture occurs in patients with a CTSS of 25 and above.[9] Similar to this situation in our study, we found a significant positive correlation between CTSS and age. Bakir et al. did not find a significant relationship between the amount of viral load detected in patients with COVID-19 pneumonia and

CTSS value.[15] Similar to this study, we did not find a significant relationship between CT score and viral load in our study.

In patients with comorbid conditions, including diabetes, chronic obstructive pulmonary disease (COPD), cardiovascular diseases, hypertension, and malignancy, COVID-19 infection has the potential to escalate into a life-threatening condition.[22] In a study conducted by Gülbudak et al., comorbid diseases were found at a higher rate in symptomatic patients compared to asymptomatic patients. According to this study, hypertension, diabetes mellitus, chronic respiratory disease and cardiovascular diseases were found most frequently in symptomatic patients.[5] According to Singh et al. chronic renal failure, diabetes mellitus, chronic liver disease, chronic lung disease and chronic cardiovascular disease were found more frequently in symptomatic COVID-19 patients.[12] Although hypertension, cardiovascular disease, diabetes mellitus and chronic obstructive pulmonary disease were more common in our study, no significant correlation was observed between the number of comorbidities and CTSS.

Our study revealed no association between COVID-19 diagnosis based on RT-PCR test Ct values and chest CT involvement as indicated by the CTSS. However, a significant correlation was observed between the CTSS and age. These findings pave the way for future in-depth researches in this domain, fostering a better understanding of the subject matter.

Conflict of Interest

The authors declare that there is not any conflict of interest regarding the publication of this manuscript.

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Ethics Committee Permission

Approval for this study was obtained from Aksaray University Clinical Research Ethics Committee (dated 16.12.2021 and numbered 2021/17-03).

Authors' Contributions

Concept/Design: AA, CÇ. Data Collection and/or Processing: DÖ, ST. Data analysis and interpretation: DÖ, CÇ, ST. Literature Search: CÇ, NBK. Drafting manuscript: NBK. Critical revision of manuscript: AA, DÖ, ST, NBK. Danışmanlık: AA, DÖ.

REFERENCES

1. Zhou M, Zhang X, Qu J. Coronavirus disease 2019 (COVID-19): a clinical update. Front Med. 2020; 14(2):126-135.
2. Trunfio M, Venuti F, Alladio F, et al. Diagnostic SARS-CoV-2 Cycle Threshold Value Predicts Disease Severity, Survival, and Six-Month Sequelae in COVID-19 Symptomatic Patients. Viruses. 2021;13 (2):281.
3. Rao SN, Manissero D, Steele VR, Pareja J. A systematic review of the clinical utility of cycle threshold values in the context of COVID-19. Infect Dis Ther. 2020;9(3):573-586.
4. Tom MR, Mina MJ. To Interpret the SARS-CoV-2 Test, Consider the Cycle Threshold Value. Clin Infect Dis. 2020;71(16):2252-2254.
5. Gülbudak H, Karvar Ş, Soydan G, et al. Comparison of Real Time PCR Cycle Threshold Values in Symptomatic and Asymptomatic COVID-19 Patients. Mikrobiyol Bul. 2021;55(3):435-444.
6. Al-Mosawe AM, Abdulwahid H Mohammed, Fayadh NAH. Spectrum of CT appearance and CT severity index of COVID-19 pulmonary infection in correlation with age, sex, and PCR test: an Iraqi experience. Egypt J Radiol Nucl Med. 2021;52(1):40.
7. Yang R, Li X, Liu H, et al. Chest CT Severity Score: An Imaging Tool for Assessing Severe COVID-19. Radiol Cardiothorac Imaging. 2020;2(2):200047.
8. Shah S, Singhal T, Davar N, Thakkar P. No correlation between Ct values and severity of disease or mortality in patients with COVID 19 disease. Indian J Med Microbiol. 2021;39(1):116-117.
9. Saeed GA, Gaba W, Shah A, et al. Correlation between Chest CT Severity Scores and the Clinical Pa-
rameters of Adult Patients with COVID-19 Pneumonia. Radiol Res Pract. 2021;2021(1): 6697677.
10. Caruso D, Zerunian M, Polici M, et al. Diagnostic performance of CT lung severity score and quantitative chest CT for stratification of COVID-19 patients. Radiol Med (Torino). 2022;127(3):309-317.
11. Markewitz R, Torge A, Wandinger KP, et al. Analysis of SARS-CoV-2 reverse transcription-quantitative polymerase chain reaction cycle threshold values vis-à-vis anti-SARS-CoV-2 antibodies from a high incidence region. Int J Infect Dis. 2021;110:114-122.
12. Singh V, Agarwal J, Garg J, SaquiB M, Das A, Sen M. Role of Cycle Threshold of RT-PCR in the Prediction of COVID-19 Cases. J Microbiol Infect Dis. 2021;11(3):132-139.
13. De la Calle C, Lalueza A, Mancheño-Losa M, et al. Impact of viral load at admission on the development of respiratory failure in hospitalized patients with SARS-CoV-2 infection. Eur J Clin Microbiol Infect Dis. 2021;40(6):1209-1216.
14. Shah VP, Farah WH, Hill JC, et al. Association Between SARS-CoV-2 Cycle Threshold Values and Clinical Outcomes in Patients With COVID-19: A Systematic Review and Meta-analysis. Open Forum Infect Dis. 2021;8(9):ofab453.
15. Bakir A, Hosbul T, Cuce F, et al. Investigation of Viral Load Cycle Threshold Values in Patients with SARS-CoV-2 Associated Pneumonia with Real-Time PCR Method. J Infect Dev Ctries. 2021;15(10):1408-1414.
16. Ayyildiz VA. Cardiothoracic Radiological Imaging and The Role of Artificial Intelligence in Covid-19. Med J SDU. 2021;28(Special issue-1):101-112.
17. Jędrusik P, Gaciong Z, Sklinda K, Sierpiński R, Walecki J, Gujski M. Diagnostic role of chest computed tomography in coronavirus disease 2019. Pol Arch Intern Med. 2020;130(6):520-528.
18. Wang Y, Dong C, Hu Y, et al. Temporal Changes of CT Findings in 90 Patients with COVID-19 Pneumonia: A Longitudinal Study. Radiology. 2020;296(2): 55-64.
19. Karahasan Yagci A, Sarinoglu RC, Bilgin H, et al. Relationship of the cycle threshold values of SARS-CoV-2 polymerase chain reaction and total severity score of computerized tomography in patients with COVID 19. Int J Infect Dis. 2020;101:160-166.
20. Francone M, Iafrate F, Masci GM, et al. Chest CT score in COVID-19 patients: correlation with disease severity and short-term prognosis. Eur Radiol. 2020;30(12):6808-6817.
21. Li K, Chen D, Chen S, et al. Predictors of fatality including radiographic findings in adults with COVID-19. Respir Res. 2020;21(1):146.
22. Ejaz H, Alsrhani A, Zafar A, et al. COVID-19 and comorbidities: Deleterious impact on infected patients. J Infect Public Health. 2020;13(12):1833-1839.