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Relationship Between Viral Load and Laboratory Values and Radiological Findings in Patients with COVID-19

COVID-19 Hastalarında Viral Yük ile Laboratuvar Parametreleri ve Radyolojik Bulgular Arasındaki İlişki

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Abstract

Introduction: This study investigates the relationship between the viral load calculated from oropharyngeal/nasopharyngeal swabs at diagnosis and the laboratory parameters and radiological findings in patients with Coronavirus disease-2019 (COVID-19).
Materials and Methods: Sixty-seven patients who were positive for severe acute respiratory syndrome-coronavirus-2 via quantitative reverse transcription-polymerase chain reaction (qPCR) from their oropharyngeal/nasopharyngeal swabs and admitted to Malatya Turgut Özal University Hospital were included in the study. Demographic data, laboratory parameters, and the severity of thorax computed tomography findings were recorded. The relationship between the viral load and these data was compared.
Results: The mean age of the patients was 63.4±9.8 years, mean body mass index (BMI) was 28.6±5.4 kg/m^2, and mean cycle threshold (Ct) values were 21.4±5.2 cycles. No correlation was found between Ct value and gender, age, and BMI. There was a significant relationship between radiological severity and Ct value, age, and gender. There was a significant correlation between the Ct value and C-reactive protein, leukocyte, neutrophil, lymphocyte, neutrophil-lymphocyte ratio, ferritin, albumin, and calcium levels. In contrast, no significant correlation was found between hemoglobin, hematocrit, thrombocyte, urea, creatinine, total protein, gamma glutamyl transferase, alkaline phosphatase, sodium, potassium, D-dimer, procalcitonin levels, and Ct value.
Conclusion: The viral load amount shown by PCR during the early period predicts the condition of the patient's lung in the advanced immunological phase. The Ct value can be an independent factor for evaluating the patient's radiological and biochemical status.
Keywords: SARS-CoV-2, viral load, lymphopenia

Öz

Giriş: Koronavirüs hastalığı-2019 (COVID-19) hastalarında tanı aşamasında orofarenks/nazofarenks sürüntüsünden hesaplanan viral yük ile laboratuvar parametreleri ve radyolojik bulgular arasındaki ilişkinin araştırılması amaçlandı.
Gereç ve Yöntem: Malatya Turgut Özal Üniversitesi Hastanesi'ne başvuran orofarenks/nazofarenks sürüntüde revers transkriptaz-polimeraz zincir reaksiyonu (qPCR) ile ciddi akut solunum yolu sendromu-koronavirüs-2 pozitif saptanan 67 hasta çalışmaya dahil edildi. Hastalara ait demografik veriler, laboratuvar parametreleri ve toraks bilgisayarlı tomografi bulguları kaydedildi. Viral yük ile bu veriler arasındaki ilişki karşılaştırıldı.
Bulgular: Hastaların yaş ortalaması 63,4±9,8, ortalama vücut kitle indeksi (VKİ) 28,6±5,4, ortalama döngü eşik değeri (Ct) 21,4±5,2 idi. Ct değeri ile cinsiyet, yaş ve VKİ arasında ilişki bulunamadı. Radyolojik bulguların şiddeti ile Ct değeri, yaş ve cinsiyet arasında istatistiksel olarak anlamlı bir ilişki vardı. Ct değeri ile laboratuvar parametreleri olan C-reaktif protein, lökosit, nötrofil, lenfosit, nötrofil-lenfosit oranı, ferritin, albümin ve kalsiyum düzeyleri arasında istatistiksel olarak anlamlı bir ilişki vardı. Hemoglobin, hematokrit, trombosit, üre, kreatinin, total protein, gama

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glutamil transferaz, alkalen fosfataz, sodyum, potasyum, D-dimer, prokalsitonin düzeyleri ve Ct değeri arasında ise istatistiksel olarak anlamlı bir ilişki bulunmadı.
Sonuç: Sonuç olarak PCR ile erken dönemde gösterilen viral yük miktarı, hastanın akciğerlerinin ileri immünolojik fazda ne kadar kötü olabileceğini öngörmektedir. Ct değeri, hastaların radyolojik ve biyokimyasal durumunu değerlendirmek için bağımsız bir faktör olarak kullanılabilir.
Anahtar Kelimeler: SARS-CoV-2, viral yük, lenfopeni

Introduction

Novel coronavirus 2019 disease (COVID-19), caused by the severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) virus, was declared a global pandemic by the World Health Organization on March 12, 2020[1]. The incubation period of COVID-19 is approximately 14 days, and symptoms are generally observed within 2-5 days after exposure[2]. Asymptomatic COVID-19 cases are contagious, and the most important place of disease involvement is the lungs. Therefore, radiological demonstration of lung involvement is critical[3]. Although the gold standard for diagnosing the disease is the real-time polymerase chain reaction (RT-PCR) test, radiological imaging is used extensively because of the inability to perform this test in some places and because of false negativity observed in the early period[4].

It has been found that the severe lymphopenia that persists at initial diagnosis and throughout the disease is associated with mortality[5]. Further, studies have shown that elevated D-dimer, serum ferritin, troponin, C-reactive protein (CRP), and liver function tests are elevated at the initial diagnosis or increase during follow-up and are essential in prognosis[6]. The neutrophil-lymphocyte ratio (N:L) and leukocyte albumin ratio are independent risk factors in evaluating disease severity[7,8].

Viral load is the amount of viral RNA in a given volume of an infectious sample. It is also referred to as the threshold number of threshold cycles or the cycle threshold (Ct) value. The Ct value represents the number of amplification cycles required for a target gene to exceed a threshold detection level. It is inversely proportional to the viral load; the lower the Ct value, the higher the viral load. Test results are considered positive when the Ct value is less than 38 in SARS-CoV-2[9,10]. This study investigates the relationship between the viral load detected in oropharyngeal/nasopharyngeal swabs at diagnosis and the laboratory parameters and radiological findings in patients with COVID-19.

Materials and Methods

Demographic data, laboratory data, and radiological parameters of patients who were admitted to the Malatya Turgut Özal University Hospital COVID-19 outpatient clinic in December 2020 were evaluated retrospectively. For our study, 100 patients with positive PCR tests were planned. The aim was to collect the radiological findings and laboratory data of patients retrospectively from the digital archive system of our hospital. However, as the data of 33 positive patients could not be accessed in the retrospective screening, they were excluded from the study. Sixty-seven RT-PCR-positive patients whose laboratory information and radiological findings were obtained were included in the study.

PCR tests were performed in the first 3 days after the patients' complaints started. That is, viral load was measured in the early viral phase. The radiological and laboratory parameters of patients were measured between 10 and 14 days (mean, 12±1.5 days) in the advanced immunological stage. The Ct values of patients were obtained by evaluating the oropharyngeal/nasopharyngeal samples with the SARS-CoV-2 double gene RT-qPCR (Bio-Speedy-USHAŞ, Ankara, Turkey) kit, and values below 38 were considered positive. Routine hematology (SYSMEX, Automated Hematology, Wakinohama-Kaigondori, Chuo-ku Kobe Japan) and biochemical tests (Architect, Toshiba, Abbott Park, USA) were recorded. Examinations included leukocyte count, neutrophil count, lymphocyte count, hemoglobin, hematocrit, thrombocyte, CRP, electrolytes, procalcitonin, D-dimer, liver/kidney function tests, and Ct values.

Thorax computed tomography findings taken at the time of the first admission of patients were evaluated. All images were acquired from a 16-slice computed tomography scanner (Philips Medical Systems, Shenyang, China). The radiological findings of patients were evaluated regarding the percentage of lobar involvement in each of the five lung lobes. Radiological involvement was obtained by summing the five lobe scores (range, 0-20) according to the total severity score and classified as normal (0%), minimal pneumonia (1-25%), mild pneumonia (26-50%), moderate pneumonia (51-75%), and severe pneumonia (76-100%)[11].

The study was conducted with the permission of Malatya Turgut Özal University Clinical Research Ethics Committee and following the Declaration of Helsinki (decision no: 2021/31, date: 13.12.2021).

Statistical Analysis

Statistical Package for the Social Sciences 25 program was used in the study. An analysis of variance test was used to compare Ct values with radiological findings. The Pearson correlation test was used to determine the strength of the relationship between

Ct values and laboratory parameters. A p value of less than 0.05 was considered significant. The power of the study was determined as 95% by the G*Power method.

Results

A total of 67 patients, 32 (47.8%) women and 35 (52.2%) men, were included in the study. The mean age of the patients was 63.4±9.8 years, and the mean body mass index (BMI) was 28.6±5.4 kg/m^2. The mean Ct values of patients were 21.4±5.2 (minimum: 7.90, maximum: 33.4). No correlation was found between Ct value and gender, age, and BMI. When the Ct values of patients were evaluated according to radiological finding severity, 29.9±1.9 were in the nonpneumonia group, 25.2±1.0 were in the minimal pneumonia group, 22.3±0.8 were in the mild pneumonia group, 18.7±0.8 were in the moderate pneumonia group, and 13.5±2.1 were in the severe pneumonia group. There was a significant correlation between radiological severity and Ct value (p<0.001). There was also a significant correlation between radiological severity and age and gender (p=0.002, p=0.013) (Table 1).

A significant relationship was found between Ct value and laboratory parameters of CRP (r=-0.345, p=0.008), leukocyte count (r=-0.304, p=0.016), neutrophil count (r=-0.630, p=0.004), lymphocyte count (r=0.326, p=0.010), N:L ratio (r=-0.457, p<0.001), ferritin value (r=-0.378, p=0.043), albumin (r=0.308, p=0.017), and calcium value (r=0.309, p=0.016). In contrast, no statistically significant correlation was found between hemoglobin, hematocrit, thrombocyte, urea, creatinine, sodium, potassium, D-dimer, procalcitonin, total protein, gamma glutamyl transferase, alkaline phosphatase, and Ct value (Table 2).

Discussion

The results revealed that a significant correlation was found between the Ct value and the prevalence of radiological findings and laboratory parameters, such as CRP, ferritin, leukocytes, neutrophils, lymphocytes, N:L ratio, albumin, and calcium.

Zhao et al.[12] evaluated the early viral load and radiological findings. They found that CT images progressed rapidly in the later stages of the disease in patients with high viral load. They emphasized the importance of radiological follow-up, especially in patients with a high viral load. Similar to our findings, He et al.[13] found that a higher viral load was associated with severity of COVID-19. On the contrary, To et al.[14] suggested no relationship between disease severity and viral load but showed a direct correlation between age and viral load.

Yu et al.[15] found that high viral load in initial sputum samples was linearly associated with the severity and risk of disease progression. Studies have shown that the viral load is higher in severe patients than in mild or asymptomatic patients[16]. A recent study showed that CRP, albumin, and neutrophil counts were highly correlated with the Ct value[17]. In various studies, increased viral load has been associated with IL-6 LDH, increased N:L ratio, and lymphopenia[18,19]. Lukas et al.[18] found that a high viral load was associated with interferon (IFN) alpha, IFN gamma, and tumor necrosis factor. Azzi et al.[19] found a

Table 1. Relationship between radiological severity index and age, body mass index, and cycle threshold values

	Normal (n=9)	Minimal (n=14)	Mild (n=15)	Moderate (n=12)	Severe (n=17)	p value
Age (years)	54.5±8.2	61.7±7.3	62.2±8.2	63.6±8.4	70.7±10.6	0.002
BMI (kg/m^2)	24.2±2.3	27.6±4.1	28.5±5.1	28.8±2.6	31.7±7.3	0.013
Ct value (cycle)	29.9±1.9	25.2±1.0	22.3±0.8	18.7±0.8	13.5±2.1	<0.001

BMI: Body mass index, Ct: Cycle threshold

Table 2. Relationship between cycle threshold values and laboratory parameters

	r	p		r	p
Hemoglobin, g/dl	-0.138	0.280	CRP, mg/dl	-0.345	0.008
Hematocrit, %	-0.177	0.170	Sodium, mmol/L	-0.003	0.983
Leukocytes, 10^3/L	-0.304	0.016	Potassium, mmol/L	-0.151	0.249
Neutrophil, 10^3/L	-0.63	0.004	Calcium, mmol/L	0.309	0.016
Lymphocytes, 10^3/L	0.326	0.010	Total protein, g/dl	0.202	0.129
N:L ratio	-0.457	<0.001	Albumin, g/dl	0.308	0.017
Platelets, 10^3/L	0.051	0.694	ALP, IU/L	-0.069	0.603
D-dimer, µg/ml	-0.262	0.215	GGT, U/L	-0.248	0.060
Ferritin, ng/ml	-0.378	0.043	Urea, mg/dl	-0.125	0.373
Procalcitonin, ng/ml	0.106	0.613	Creatinine, mg/dl	0.422	0.405

N:L: Neutrophil-lymphocyte, CRP: C-reactive protein, ALP: Alkaline phosphatase, GGT: Gamma glutamyl transferase

statistically significant correlation between Ct value and markers of inflammation and between hematological markers and Ct value. In line with our study, Chen et al.[20] found that patients with a high viral load in COVID-19 had lymphopenia and increased neutrophils.

A systematic review concluded that lower Ct values might be associated with worse outcomes. Early viral load detection, especially in susceptible and elderly patients, is essential for initiating neutralizing antibody therapy, antiviral therapy, and corticosteroid therapy[21].

According to the results of our study, we found that as the viral load increased, radiological findings worsened in patients with COVID-19; laboratory parameters such as CRP, ferritin, leukocytes, neutrophils, N:L ratios increased; and albumin, calcium, and lymphocyte counts decreased.

Study Limitations

The most important limitation of our study was that it was a single-center, cross-sectional study. Our findings should be supported by prospective, controlled studies with more patients.

Conclusion

The viral load amount shown by PCR during the early phase predicts the condition of the patient's lung in the advanced immunological phase. The Ct value can be an independent factor in evaluating the patient's radiological and biochemical status. Analyzing the viral load with large-scale, prospective studies can guide current and future pandemic and infection control policies.

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Ethics

Ethics Committee Approval: Malatya Turgut Özal University Hospital Ethics Committee and following the Declaration of Helsinki (decision no: 2021/31, date: 13.12.2021).

Informed Consent: Retrospective study.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: A.A.G., Ş.A., M.A., Design: A.A.G., M.A., Data Collection or Processing: I.G.B., Ş.A., M.A., Analysis or Interpretation: A.A.G., I.G.B., M.A., Literature Search: A.A.G., Ş.A., Writing: A.A.G., Ş.A.

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Conflict of Interest: The authors declare that they have no conflict of interest.

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