



ORIGINAL ARTICLE

SARS-Cov-2 Viral Load as an Indicator for COVID-19 Patients' Hospital Stay

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Received date: January 13, 2021; **Accepted date:** March 4, 2021; **Published date:** June 30, 2021

Abstract

Background/objective: The novel coronavirus disease 2019 (COVID-19) pandemic has posed a global threat to public health. An objective strategy is quintessential to handle resources prudently. This study assessed the utility of the quantitative polymerase chain reaction (qPCR) cycle of threshold (Ct) to evaluate the severity of the COVID-19 infection. The study also assessed the duration of the hospital and the management of healthcare resources.

Methods: This cross-sectional study was carried out among 306 patients admitted to COVID-19 care centers in the Kingdom of Bahrain from 20th March 2020 to 5th April 2020. Standard qPCR was used to estimate the viral load, and data was analyzed to investigate the relationship between Ct values and various variables.

Results: Out of 306 patients, two fatalities, one active stable case, and 303 recovered cases were reported. Ct value was negatively associated (P value <0.001) with the length of hospital stay. Viral clearance was also inversely associated with the Ct values.

Conclusion: Ct value was inversely associated with the duration of hospital stay (and time to viral clearance); the higher Ct value was indicative of a faster time to viral clearance. This association could aid in the management of both the infection and resources.

Keywords: Bahrain; COVID-19; Length of Stay; Public Health; Resource Allocation; Viral Load

Introduction

Coronavirus disease 2019 (COVID-19) is caused by the newly identified strain of the Coronavirus

family Severe Acute Respiratory Syndrome Corona virus 2 (SARS-CoV-2).¹ While some patients may have mild symptoms, others develop a severe

respiratory distress syndrome with features of a blood coagulopathy requiring intensive support.² As a result, COVID-19 has rapidly evolved into a pandemic that has put a severe burden on the healthcare services of some countries, specifically beds for both hospitalization and intensive care facilities.³ Testing aids diagnosis, case isolation, and contact tracing, all of which are vital to limit coronavirus spread.⁴ Currently, the primary diagnostic test for SARS-CoV-2 is the real-time Reverse Transcription Polymerase Chain Reaction (RT-PCR).⁵ RT-PCR result is influenced by the Cycle Threshold (Ct-Value) that has been suggested as a quantitative test to indirectly assess the viral load with severe cases have a much higher viral load than mild cases.⁶ Therefore, the present study measured the viral load determined by the Ct value and correlated with viral clearance. Based on these measurements, the study also predicted the length of hospital stay and assisted prudent healthcare resource allocation.

Methods

Patients and Sample Collection

A cross-sectional study was undertaken on 306 patients who were admitted to COVID-19 care centers in the Kingdom of Bahrain between 20th March 2020 and 5th April 2020. Data was recorded for demographics, Ct-value on admission, time to viral clearance, and data on the length of stay and patient outcomes. At the time of the study, the local protocol in Bahrain admitted all positive cases to treatment/isolation facilities regardless of being symptomatic or asymptomatic. The patients were discharged when they had two negative nasopharyngeal and oropharyngeal swabs on two consecutive days. All admitted patients were tested after three days of being asymptomatic and, if negative, the test was repeated after 24 hours to confirm viral clearance, following which the patients were discharged. Therefore, the length of stay was the same as the time to viral clearance, and the minimum stay of patients was four days.

Inclusion and exclusion criteria

Cases diagnosed with COVID-19 based on PCR results were included in the study. Given that the study analyzed viral clearance and length of stay,

only recovered COVID-19 cases were included. COVID-19 fatalities were excluded as these patients did not clear the virus and could not be included in the analysis. Active cases were excluded as these cases remained infected and did not clear the virus.

RNA Extraction and SARS-Cov-2 RNA Amplification

The diagnostic test used for SARS-CoV-2 was performed using quantitative polymerase chain reaction (qPCR) on nasopharyngeal and oropharyngeal samples. The samples were transferred to a viral transport media immediately after collection and transported to a COVID-19 laboratory for testing. Diagnosed cases were a heterogeneous sample of tested patients, and they included symptomatic and asymptomatic individuals with recent exposure to a confirmed COVID19 case or travelers. PCR test was conducted using Thermo Fisher Scientific (Waltham, MA) TaqPath 1-Step RT-qPCR Master Mix, CG on the Applied Biosystems (Foster City, CA) 7500 Fast Dx Real-Time PCR Instrument. The assay used followed the WHO protocol from Charité Virology, Berlin, Germany, and targeted the E gene.⁷ If the E gene was detected, the sample was then confirmed by RdRP and N genes. The E gene Ct value was reported and used in this study. Ct Values >40 were considered negative. Positive and negative controls were included for quality control purposes. In case of inconclusive results, the sample were re-analyzed. In cases where inconclusive results persisted, the physician was notified in order to repeat sampling and testing.

RT-qPCR Analytical Validation

Linear range was determined using serial log₁₀ dilutions of standard RNA and was established between 3.5×10^3 to 3.5×10^{12} copies of RNA/mL, with amplification efficiency of 87% and with a regression R² value higher than 0.99.

Statistical Analysis

Categorical parameters were expressed as frequencies (%), whereas continuous variables were presented as mean. Data trends were visually and statistically evaluated for normality. Regression analysis was used to describe the relationship

between categorical variables and between continuous variables with the main outcome. Two-way Student's t-test was then used to detect the difference between Ct values across symptomatic and asymptomatic individuals. Statistical analysis was performed using STATA statistical computer package (StataCorp. 2013. Stata Statistical Software).

Ethics Approval

The protocol and manuscript for this study were reviewed and approved by the National COVID-19 Research and Ethics Committee in Bahrain. This committee has been jointly established by the Ministry of Health and Bahrain Defense Force Hospital research and ethical committees in response to the pandemic to facilitate and monitor COVID-19 research in Bahrain. All methods and retrospective analysis of data was approved by the National COVID-19 Research and Ethics Committee and carried out in accordance with the local guideline and ethical guidelines of the Declaration of Helsinki 1975. Informed consent was waived by the National COVID-19 Research and Ethical Committee for this study due to its retrospective and observational nature and the absence of any patient identifying information

Results

Of the 306 patients, 303 had recovered, whilst two deceased cases and one active stable case were excluded from the analysis.

The descriptive summary of the patients is shown in Table 1. Males represented the majority of cases, accounting for 71% of the sample. The mean age of the sample was 37.1 years (± 15.1). About 12.2% of the cases were symptomatic patients. About 64% of cases were secondary to local transmission, and 36% were due to travel-related infection. About 58.4% of the sample were Bahraini nationals, and 41.6% were non-Bahraini. The mean Ct value was 27.5 (± 5.1), with 89.4% of cases having a Ct value of less than 35. The mean length of stay was 11.3 days (± 5.07). Ct values showed a statistically significant negative correlation of 0.28 with the length of hospital stay and time to viral clearance as seen in Figure 1, showing the Lowess smoothing curve of hospital stay. Higher Ct values on admission

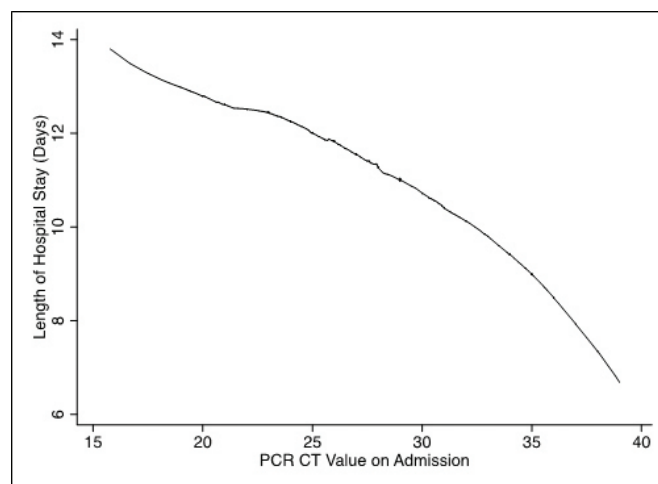


Figure 1: Lowess smoothing curve plotting length of stay (days) vs. qPCR Ct value on admission

Table 1: Characteristics of patients in the study

Variable	N	%	
Gender	Male	215	71.0%
	Females	88	29.0%
Symptoms on Admission	Asymptomatic	266	87.8%
	Symptomatic	39	12.2%
Mode of Transmission	Local	194	64.0%
	Travel	109	36.0%
Nationality	Bahraini	177	58.4%
	Indian	89	29.4%
	Bangladeshi	19	6.3%
	Other	18	5.9%
Category of Ct values	Low: Ct<35	271	89.4%
	High: Ct \geq 35	32	10.6%
Variable	N	Mean	Range
Age (years)	303	37.1 \pm 15.1	0 to 87
Ct value on Admission	303	27.5 \pm 5.1	14.8 to 39
Length of Stay (days) (Time to viral clearance in Days)	303	11.3 \pm 5.07	4 to 32

predicted shorter hospital stays and shorter time to clear the virus. The regression analysis showed similar results when using a univariate analysis and a multivariate analysis adjusted for age and gender. Ct value had a significant inverse correlation with the length of stay, 10 points higher on the Ct value correlating with a shorter hospital stay of 2.8 days.

Patients with high Ct values defined as ≥ 35 had shorter hospital stays, by 4.3 days, when compared to patients with Ct values < 35 on admission.

Table 2: Regression analysis for the length of stay using Ct value as a covariate

Multivariate regression output, Adjusted for Age and Gender				
Outcome: Length of Stay in Days				
Continuous covariate	Coefficient	Standard Error	P value	95% Confidence Interval
Ct value	-0.28	.056	<0.001*	-0.39 to -0.17
Multivariate regression output, Adjusted for Age and Gender				
Outcome: Length of Stay in Days				
Categorical covariate	Coefficient	Standard Error	P value	95% Confidence Interval
Low Ct value (<35)	-	-	-	-
High Ct value (≥35)	-3.9	0.94	<0.001*	-5.75 to -2.05

**Statistically significant*

The regression analysis for “Length of Stay” using Ct value as a covariate is shown in Table 2. Analysis on symptomatic individuals showed that symptomatic individuals have a lower mean Ct value when compared to asymptomatic individuals. Asymptomatic individuals had a mean Ct value of 27.8. Symptomatic individuals had a mean Ct value of 25.5. A Two sample T-test showed a difference of 2.58 units in the mean Ct value between symptomatic and asymptomatic patients (Table 3).

Table 3: Two-sample t test comparing mean Ct values between symptomatic and asymptomatic individuals

Status	Mean Ct value	Standard Error	95% Confidence Interval
Asymptomatic	27.8	0.31	27.2 to 28.4
Symptomatic	25.5	0.82	23.9 to 27.8
Difference	2.3	0.88	0.6 to 4.0
P Value			0.01*

**Statistically significant*

Discussion

This study has shown that the higher the Ct values, the more rapid the viral clearance, leading to a shorter length of stay in hospital. A Ct value >35 predicted a shorter hospital stay by 4.3 days, and a 10 unit increase in Ct value was associated with a shortened time to viral clearance of 2.8 days. Ct values have been reported as an indirect assessment of the viral load, with higher Ct values having a lower viral load, and it has been shown that those patients with more severe diseases have lower Ct values.^{6,8} Higher Ct values indicate an early infection or a recovering infection. Whilst Ct values can be used

to predict the length of hospital stay for stable cases, the Ct value cannot, however, predict outcomes. Outcomes are influenced by many additional predictors, most notably comorbidities and severity of the disease.⁹ The presence of age greater than 60 and comorbidities including diabetes, hypertension, obesity, and respiratory disease may offset high Ct values, and further studies to predict outcomes based on Ct value taking into account adverse risk factors of disease severity are needed.

In this study, symptomatic individuals had lower Ct values whilst asymptomatic individuals had higher Ct values, suggesting that the lower Ct values reflect a more severe presentation. However, asymptomatic individuals still had low mean Ct values (27.8), indicating a significant viral load, suggesting that asymptomatic individuals can still transmit the disease.¹⁰ These results are in accord with those of Liu et al., who reported that the mean viral load of severe cases was around 60 times higher than that of mild cases.⁶ La Scola et al. have reported using a cell culture system that patients with Ct values ≥34 do not shed infectious virions, and hence are not infectious and can be discharged from hospitals or care centers.¹¹

Bahrain has pursued a comprehensive testing program with all travelers arriving in the country being tested, mobile screening services, random screening in high-risk communities, and extensive contact tracing. This strategy has resulted in detecting many asymptomatic patients, in contrast to other countries where the response has been directed solely towards symptomatic patients and has generated data that is critical for the production of accurate prediction models.¹²

Limitations of this study include that it was cross-sectional and that the number of symptomatic cases were few in number. The number of patients with multiple comorbidities were few, and therefore, a model to consider the protective effect of a high Ct value could not be determined.

Conclusion

In this current study, it was evident that the Ct value can predict the duration of hospital stay of COVID-19 infected patients, which also suggests that viral clearance will be faster in higher Ct value patients. This data can be implemented in other countries to evaluate the patients' stay at the hospital. This model would aid in allotting adequate healthcare staff in successfully handling the COVID-19 patients.

Declarations

Ethics approval and consent to participate: The study was approved by the National COVID-19 Research and Ethics Committee.

Consent for publication: Consent for publication was waived by the Ethics committee.

Availability of data and materials: All the data for this study will be made available upon reasonable request to the corresponding author.

Funding: No funding was received to perform this study.

Conflict of interest: The authors have declared that no conflict of interest exists.

Author contributions: SA and OY analyzed the data and wrote the manuscript. AA, OY, and SA contributed to study design, collected, analyzed, and interpreted data, and edited the manuscript. MA supervised data collection, data analysis and edited the manuscript. EMJ data interpretation and the editing of the manuscript. All authors reviewed and approved the final version of the manuscript. Manaf Alqahtani is the guarantor of this work.

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