RESEARCH ARTICLE

High proportion of low cycle threshold value as an early indicator of COVID-19 surge

Baijayantimala Mishra ⓘ | **Jai Ranjan** ⓘ | **Prashanth Purushotham** ⓘ |
Swarnatrisha Saha | **Poesy Payal** | **Punyatoya Kar** ⓘ | **Sivasankar Das** |
Vaishnavi Deshmukh

Department of Microbiology, All India
Institute of Medical Sciences, Bhubaneswar,
Odisha, India

Correspondence
Baijayantimala Mishra, Department of
Microbiology, All India Institute of Medical
Sciences, Bhubaneswar, Odisha 751019, India.
Email: bm_mishra@hotmail.com

Abstract

Many countries in the world are experiencing a recent surge in COVID-19 cases. This is mainly attributed to the emergence of new SARS-CoV-2 variants. Genome sequencing is the only means to detect the evolving virus mutants and emerging variants. Cycle threshold values have an inverse relationship with viral load and lower Ct values are also found to be associated with increased infectivity. In this study, we propose to use Ct values as an early indicator for upcoming COVID-19 waves. A retrospective cross-sectional study was carried out to analyze the Ct values of positive samples reported during the first wave and second wave (April 2020–May 2021). Median Ct values of confirmatory genes were taken into consideration for comparison. Ct values below 25, >25–30, and >30 were categorized as high, moderate, and low viral load respectively. Our study found a significantly higher proportion of positive samples with a low Ct value (<25) across age groups and gender during the second wave of the COVID-19 pandemic. A higher proportion of positive samples with a low Ct value (high viral load) may act as an early indicator of an upcoming surge.

KEYWORDS
COVID-19, cycle threshold, SARS-CoV-2, surge, variants

1 | INTRODUCTION

The high transmissibility of the SARS-CoV-2 virus has led to the pandemic of COVID-19. Ever since the declaration of the COVID-19 pandemic on March 11, 2020, the entire world continues to be under the grip of the virus. Many places in the world including India have already experienced two waves of the pandemic. In India, the second wave started in the month of April 2021 with an unprecedented rapid surge of cases which almost crippled the health care system. The second wave in India is mostly attributed to B.1.617, which has been shown to be more infectious as compared to the initial wild-type SARS-CoV-2 strain.[1] However, by the time the role of variant came into the limelight, the country had already suffered damage in terms of widespread infection, high hospitalization, and high mortality. Genome sequencing is in place to keep track of the evolving virus mutants and emerging variants. However, keeping pace with real-time and adequate sampling is a big challenge in the present pandemic scenario and more so for the resource-constrained countries.[2]

Real-time polymerase chain reaction (RT-PCR) is the mainstay of COVID-19 diagnosis. The cycle threshold (Ct) value represents the polymerase chain reaction cycle at which the fluorescence signal crosses the defined background threshold. Higher the target concentration, earlier the signal crosses the threshold giving a lower Ct value and vice versa for the low target concentration. Therefore, the Ct value is considered as a surrogate marker of the virus load and is inversely related to the viral load.[3] Several studies have linked the

low Ct value with the infectiousness of the SARS-CoV-2 virus.[4,5] As the property of infectiousness is linked to transmissibility and consequently the rapid surge of cases, it is important to find the possible role of Ct value of COVID-19 RT-PCR as an early marker of the surge of COVID-19 positive cases, particularly if the surge is going to be due to a different variant. With this objective, the present retrospective study was planned to analyze the Ct values across different sections of cases during the first and second waves of the COVID-19 pandemic.

2 │ METHODOLOGY

The study was conducted in the Department of Microbiology, All India Institute of Medical Sciences (AIIMS), Bhubaneswar, which is a designated COVID-19 RT-PCR laboratory by the Indian Council of Medical Research (ICMR), Government of India, and a tertiary care hospital in Eastern India, which received samples from different districts of Odisha as well as from suspected COVID-19 cases attending the screening clinic or admitted in COVID ward.

A retrospective cross-sectional study was carried out to analyze the Ct values of positive samples reported during the first wave and second wave (April 2020–May 2021) from our laboratory. Ct values across different sections of samples were compared; namely between different age groups, gender, and patients with and without symptoms. Results from 13 816 RT-PCR positive samples consisting of 9308 that occurred during the first wave of 2020 and 4508 during the second wave of 2021 were included for analysis of Ct values.

Median Ct values of confirmatory genes; namely ORF/RdRp and N genes were taken into consideration for comparison. Ct values below 25, >25–30, and >30 were categorized as high, moderate, and low viral load, respectively. The study was approved by the Institutional Ethics Committee of AIIMS Bhubaneswar (IEC: T/IM-NF/Micro/21/41).

The data was analyzed using Microsoft Excel (Microsoft Office 2016, Microsoft Corporation) and SPSS windows version 25.0 (IBM Corporation). The Ct values among different groups of cases were analyzed for normality using the Shapiro–Wilk test. If data was normal, an independent t-test was used to calculate the mean difference between the groups. Mann–Whitney U-test was used to compare medians between two groups in case of skewed data. $p < 0.05$ was considered statistically significant.

3 │ RESULTS

The peak positivity was recorded during the month of September 2020 and May 2021 for the first and second waves, respectively (Figure 1).

As the number of cases varied widely across the months in both the waves, the data was represented as the median and interquartile range (IQR). Median Ct values (IQR) of cases in 2020 and 2021 were 29 (22, 32) and 23 (18, 29). Figure 2 depicts the median Ct value of positive cases during 2020 and 2021. The percentage of COVID-19 positives with high viral load (≤25 Ct value) during years 2020 and 2021 was 35.7% and 60%, respectively. The difference was found to be significant ($p < 0.001$). However, the percentage of moderate (Ct: >25 to ≤ 30) and low viral load (Ct: >30) during 2020 and 2021 was 22.7% versus 19.6% and 41.6% versus 20.4%, respectively (Table 1).

To explore the difference in Ct value gender-wise and in different age groups, we compared the Ct value in these groups.

The cycle threshold values of males and females were found to be comparable in 2020 and 2021 (Figure 3). However, when the median Ct value in both the genders was compared year-wise between 2020 and 2021 the difference was found to be significant (median Ct 29 vs. 23), as shown in Figure 3.

We tried to look for the Ct value difference across different age groups during both the waves and the Ct value difference in each age group during the first and second waves. The median Ct value difference during the first wave and the second wave in each age group was found to be significant (Figure 4).

3.1 │ Symptomatic versus asymptomatic

On comparison of COVID-19 positive symptomatic and asymptomatic individuals from April 2020 to May 2021, the median Ct value for symptomatic individuals was 24.79 (IQR: 19–31) and for

FIGURE 1 Month wise COVID-19 positive cases
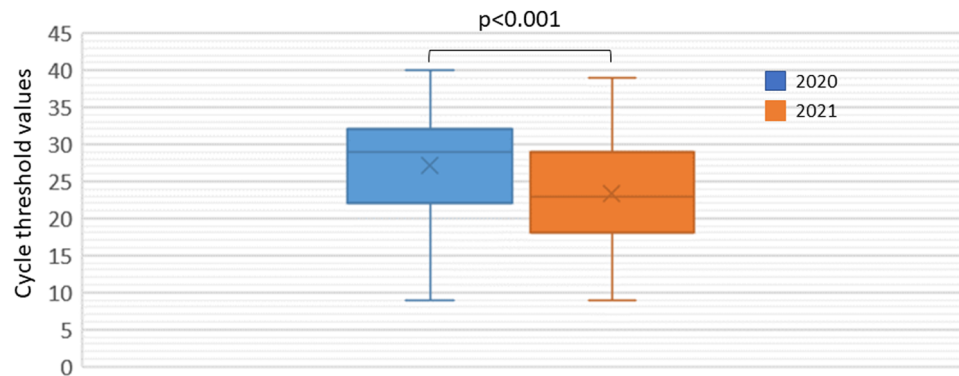
FIGURE 2   Ct values of COVID-19 positive cases during 2020 and 2021. Ct, cycle threshold

TABLE 1   Percentage and median IQR of Ct values of the first wave and second wave

Ct values	2020 (n = 9308)	2021 (n = 4508)	p
Below 25	3326 (35.7%)	2706 (60%)	p < 0.001
25–30	2116 (22.7%)	883 (19.6%)	p < 0.001
>30	3864 (41.6%)	919 (20.4%)	p < 0.001
Median (IQR)	29 (22–32)	23 (18–29)	

Abbreviations: Ct, cycle threshold; IQR, interquartile range.

asymptomatic was 26.83 (IQR: 21–32) with a significant difference in Ct value between the groups ($p < 0.001$; Figure 5).

The median Ct values of symptomatic and asymptomatic cases during the first wave (2020) were 25.85 (IQR: 20–32) and 28.27 (IQR: 23–33), and during the second wave were 21.98 (IQR: 17–27) and 23.98 (IQR: 18–30), respectively (Figure 5).

3.2 | High proportion of low cycle threshold values during the second wave

Figure 6 depicts the percentage of Ct values among the positive cases during the first and second waves of COVID-19 infection. Ct values >30 were predominantly seen during the first wave; while the proportion of Ct values <25 was significantly higher before the peak of the second wave of infections.

4 | DISCUSSION

Our study found a significantly higher proportion of positive samples with a low Ct value (<25) as a proxy of high viral load during the second wave as compared to the first wave of the COVID-19 pandemic. The cycle threshold value is the number of polymerase chain reaction cycles at which the fluorescence signal of a particular sample crosses the defined threshold. Ct value has an inverse relationship with the amount of virus present in a particular sample. A higher Ct value indicates a lower viral load in the sample and vice versa. Doubling of viral load results in a single point difference in Ct value. Thus, a 3.3 rise in Ct value indicates a ten folds decrease in viral load.[6,7] Ct values being a proxy of viral load can also serve as an indicator for infectivity and severity of a disease. Jaafar et al.[8] in their study have also shown that a sample with a lower Ct value has higher infectivity. Our study found a significantly lower Ct value during the 2021 second wave as compared to that of the first wave that occurred in 2020; with Ct values <25 being observed in over 50% of positive cases before the peak of the second wave (Figure 6). The higher proportion of high viral load during the second wave could have been attributed to the rapid surge of cases. A recent study by Hay et al.[9] has suggested that a decrease in the median cycle threshold value of a population may indicate increased transmission. The surge of the second wave in India was more abrupt indicating the possibility of transmission due to a more infectious virus variant which was confirmed afterward due to the predominant circulation of B.1.617, Indian variant, now named as Delta variant of concern by the World Health Organization and shown to be more infectious.[10,11] Significantly high proportion of positive cases with high viral load (low Ct) may be an early indicator of the pandemic surge. The median Ct value has also been suggested as a useful marker for predicting a pandemic surge.[6] Zein et al.[12] have reported a progressive decline in percentage positivity of high and intermediate viral load as the pandemic progresses along with the increase in the proportion of positive samples with low viral load indicating an upward trend of Ct value as the pandemic surge attenuates.

The decrease in the median Ct values of the second wave was approximately 3 points less than that of the first wave across different age groups of positive cases, indicating that SARS-CoV-2 cases in the second wave were harboring about 10 times higher viral load, making them more infectious than the SARS-CoV-2 of the first wave.

Several other studies have reported lower Ct value among symptomatic as compared to the asymptomatic individuals as observed in the present study.[7,13] This lower Ct value among the symptomatic individuals could be because of the presence of higher viral loads among them. As the disease progresses, the viral load decreases, and hence asymptomatic individuals can have a higher
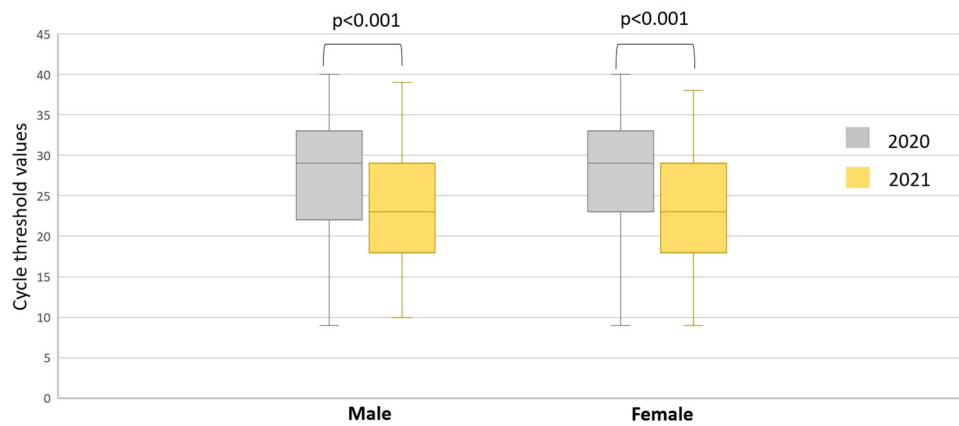
FIGURE 3 Gender wise Ct value comparison in 2020 versus 2021. Ct, cycle threshold
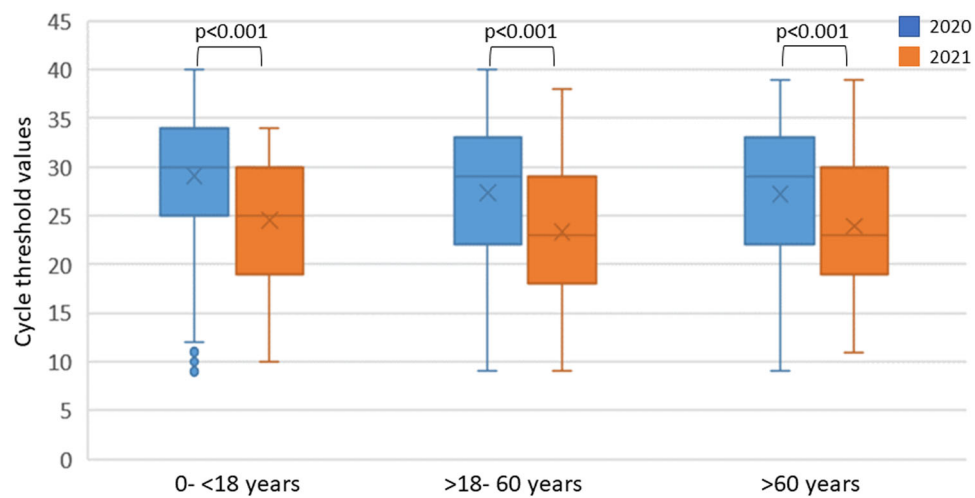

FIGURE 4 Comparison of Ct value across age groups (confirmatory genes) 2020 and 2021. Ct, cycle threshold
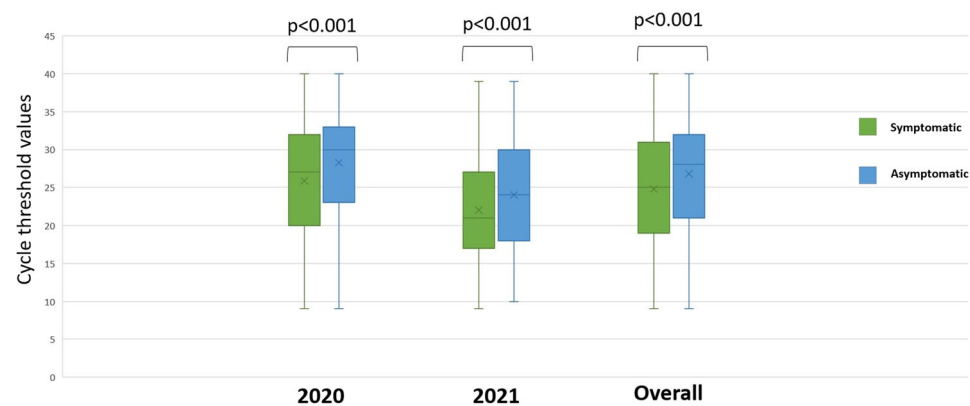
FIGURE 5 Ct value of symptomatic and asymptomatic cases. Ct, cycle threshold

Ct value. In contrast, studies by Lee et al.[14] and Louie et al.[15] found comparable Ct between asymptomatic and symptomatic cases. A larger longitudinal study is required to assess whether there exists a correlation between cycle threshold values and symptoms.

A comparison of 2020 and 2021 cycle threshold values of symptomatic and asymptomatic cases (Figure 5) shows a significant decrease in Ct value amongst the symptomatic and asymptomatic cases of 2021 compared to 2020 values. Thus, a
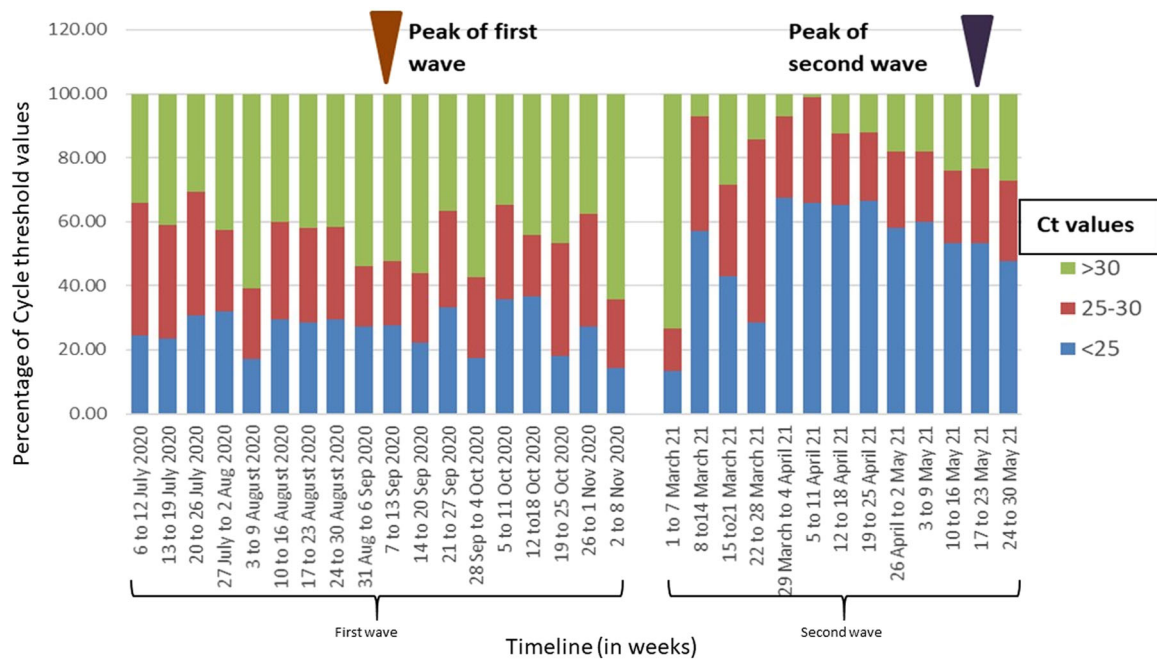
FIGURE 6 Comparison of the proportion of weekly Ct values in the first and second waves of COVID-19. Ct, cycle threshold

lower Ct value is uniformly seen among both the groups during the second wave.

Ct value depends on several variables such as type and quantity of biological material, target genes, normalization of RT-PCR assay, different RT-PCR kits, and RT-PCR instruments, and so on. Therefore, the use of Ct value in a clinical setup for individual patient management purposes has been criticized.[16] However, the use of Ct values across population or locality has been shown to be a useful indicator of COVID-19 dynamics in the community.

5 | CONCLUSION

Our study highlights the importance of Ct value analysis on a population or locality basis. The higher proportion of positive samples with a low Ct value (high viral load) may act as an early indicator of an upcoming surge.

With the continuance of pandemics and the emergence of virus variants, it is imperative to keep track of the virus and its behavioral pattern so that timely preventive action can be taken. Real-time genomic surveillance has failed to a large extent to predict the upcoming pattern of the pandemic surge, particularly in resource-limited countries and countries with a high population burden. Real-time PCR being the most widely used diagnostic test for COVID-19, the observation of the pattern of Ct value by microbiologists has the potential to act as a readily available tool to give a signal of the upcoming pandemic surge.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Baijayantimala Mishra: Concept, Design, Analysis, Drafting, and Finalization of manuscript. Jai Ranjan: Contributed to designing of study, Data Analysis, Drafting of manuscript. Prashanth Purushotham: Contributed to designing of study, Data analysis, Drafting of manuscript. Swarnatrisha Saha, Poesy Payal, Punyatoya Kar, Sivasankar Das, and Vaishnavi Deshmukh: Contributed to data acquisition and Finalizing the manuscript. All authors have approved the final version of the manuscript.

ORCID

Baijayantimala Mishra https://orcid.org/0000-0002-2604-6678
Jai Ranjan http://orcid.org/0000-0002-6229-1240
Prashanth Purushotham http://orcid.org/0000-0001-6615-7973
Punyatoya Kar http://orcid.org/0000-0002-1015-2038

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