

Evaluation of the E gene RT-PCR Ct Values in Clinical Samples from Symptomatic and Asymptomatic COVID-19 Patients

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Abstract

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has spread globally and is a major public health issue. Procedures that have been established to decrease the spread of the virus depend on the careful and precise detection of infected individuals using quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR). There have been many ambiguous concerns among the public regarding the severity of infection and its connection with the Cycle threshold (Ct) value and there was forceful need to inform the values to the public especially those with symptoms. The main objective of this study was to determine the association between the E (Envelop) gene Ct values of the symptomatic and asymptomatic COVID-19 patients. The study was conducted at the Virus Research and Diagnostic Laboratory (VRDL), Jagdalpur, Chhattisgarh. Between March 2020 and June 2020, samples were collected from the Bastar region as per the Indian Council of Medical Research (ICMR) guidelines. A total of 29228 clinical samples were tested by qRT-PCR targeting the E and RdRp (RNA-dependant RNA polymerase) genes as well as the ORF (open reading frame) gene that encode polyproteins of SARS-CoV-2. Of the 29228 samples tested, 75 were tested positive and 29153 were tested negative. In addition, the Ct values varied between the symptomatic and asymptomatic patients. It was observed that, the Ct values ranged from 15 to 32 in the asymptomatic patients and between 13 to 34 in the symptomatic patients. E gene Ct value analysis showed no significant difference between the asymptomatic and symptomatic COVID-19 patients. Thus, we observed that there was no association between the Ct values of symptomatic and asymptomatic patients of COVID-19.

Keywords: Coronavirus, SARS-CoV-2, COVID-19, E gene, symptomatic, asymptomatic, Ct value

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INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) which is the causative agent of the ongoing coronavirus disease 2019 (COVID-19) pandemic is a new zoonotic coronavirus that was discovered in the Hubei Province, China in December 2019. The World Health Organization (WHO) declared the COVID-19 pandemic, a Public Health Emergency of International Concern^{1,2}. The most prevalent COVID-19 symptoms include fever, normal to decreased leukocyte count and ground glass opacities on the computed tomography (CT) scan^{3,4}. Symptoms such as headaches, hemoptysis, abdominal pain, diarrhea, and the production of sputum have also been observed in some patients³. Currently, the two foremost techniques available for the diagnosis of SARS-CoV-2 infection is the detection of viral RNA or the measurement of the antibody produced upon an exposure to the virus. Viral RNA is detected by qRT-PCR designed to amplify the S (spike), E, N (nucleocapsid), RdRp (RNA-dependant RNA polymerase) and ORF1a/b (Open Reading Frame 1a/b) genes more frequently^{5,6}. There have been many ambiguous concerns among the public regarding the severity of infection and its connection with the Cycle threshold (Ct) value and there was forceful need to inform the values to the public especially those with symptoms. The main objective of this study was to determine the association between the E (Envelop) gene Ct values of the symptomatic and asymptomatic COVID 19 patients. The current study is focused on analyzing the association between the Ct values of the symptomatic and asymptomatic patients with COVID-19.

MATERIALS AND METHODS

Sample Collection

The study was conducted at the Directorate of Health Research (DHR)/ICMR VRDL, Late Baliram Kashyap Memorial Government Medical College (LBKM GMC), Jagdalpur, Chhattisgarh. Samples were collected from various collection centers in the Bastar region from March - 2020 to June - 2020 and categorized according to the ICMR guidelines for COVID-19. As per the ICMR guidelines, qRT-PCR positive patients were admitted to the GMC Hospital and were prescribed Hydroxychloroquine (HCQ), zinc sulfate, vitamin B complex and vitamin

C. Symptomatic patients were prescribed oxygen and medications as described above.

RNA Extraction

Nasopharyngeal and oropharyngeal samples were processed and viral RNA was extracted using the Qiagen - QIAmp Viral RNA Mini Kit.

RT-PCR Analysis

RNA was subjected to qRT PCR analysis using the AgPath ID™ One Step RT-PCR kit (Invitrogen) and the ABI™ Fast 7500 Real-Time PCR system. For the positive samples, Ct values for the E, ORF and RdRp qRT-PCRs were uploaded to the ICMR COVID-19 data portal. E gene Ct values (within the positivity range) were used to categorize viral loads: high viral load (13 to less than 20), moderate viral load (20 to less than 30) and low viral load (greater than 30) respectively. Samples with a Ct value greater than 35 were considered negative for SARS-CoV-2 and excluded from the analysis.

Statistical Analysis

The data were analyzed by EPI INFO™ version 7 Epi Info™ website (<http://wwwn.cdc.gov/epiinfo/>). 2 x 2 contingency table analysis was done and significance of tests was decided at P value less than 0.05.

RESULTS

A total of 29228 patient samples were tested by qRT-PCR. Of these, 75 patients tested positive and were admitted to the GMC hospital. Of the 75 positives, 32 patients were symptomatic and 43 were asymptomatic. Men made up 92% of the positive patients. The mean age observed for the symptomatic and asymptomatic patients was 32.69 and 29.97 years, respectively. In the asymptomatic patients, the Ct values of the E gene qRT-PCR ranged between 15 to 32, whereas in the symptomatic patients, these Ct values ranged from 13 to 34, (Table 1).

Table 1. Ct values of E gene among the symptomatic and asymptomatic patients

Category	Ct values of E gene(Range)		
	13 - < 20	20 - 30	>30
Asymptomatic	14/43	24/43	5/43
Symptomatic	16/32	12/32	4/32

Table 2. 2 x 2 contingency table for Ct values (>30 and <30) of E gene

Category	Ct values of E gene		Total	P value
	>30	<30		
Asymptomatic	5	38	43	P = 0.908 (> 0.05)
Symptomatic	4	28	32	
Total	9	66	75	

Table 3. 2 x 2 contingency table for Ct values (>20 and <20) of E gene

Category	Ct values of E gene		Total	P value
	>20	<20		
Asymptomatic	31	12	43	P = 0.43 (> 0.05)
Symptomatic	20	12	32	
Total	51	24	75	

Using a 2 x 2 contingency table, no significant difference was observed between the symptomatic and asymptomatic patients with COVID-19 (Table 2) when comparing the E gene the Ct values (>30 and <30). Similarly, the Ct values (>20 and <20) of the E gene showed no significant difference between the symptomatic and asymptomatic patients (Table 3).

DISCUSSION

It is well observed that there is a correlation between the Ct value and viral load, which could be detected as early as day 1 and peaks during the first week after the symptoms start⁷. A Ct value is the amplification cycle which the fluorescent signal exceeds that of the background signal and thus passes the threshold for positivity; therefore low Ct values signify a high viral RNA load⁸. It is understood that Ct values are not associated with the disease severity; however, it has been observed that it is inversely proportional to the viral load and hence the transmission⁸⁻¹⁰. Recently the patients have been requesting the Ct values of their COVID-19 qRT-PCR tests to correlate this with the outcome of their infection. Our study shows that there is no correlation between Ct values and outcome of the infection. However, in this study we could not take all the samples on the same day from the patients displaying symptoms. Therefore, there is no uniformity in

the Ct values obtained for different samples during the period of infection, and thus Ct values could not be compared. Although it has been suggested that wearing a mask decreases the inoculum of the virus and may minimize the progression to serious hypoxemia and acute respiratory distress syndrome (ARDS), qRT-PCR cannot predict a viral load that may result in disease progression. We know that ARDS and pneumonia in COVID-19 are influenced by other factors such as immunity, inflammation, hypercoagulopathy, oxidative stress and other comorbid conditions, including cardiovascular disease, hypertension, diabetes and obesity. Alongside RT-PCR results, biomarkers such as C-reactive protein, ferritin, D-dimer and CT scans should be included in disease prognosis. In the present study, we observed that there was no significant difference between the Ct values obtained for the E gene between the symptomatic and asymptomatic patient groups. Therefore, we conclude that it is not possible to predict the clinical outcome of COVID – 19 disease from RT-PCR Ct values.

CONCLUSION

COVID-19 is a comparatively new disease, and all avenues must be investigated in order to determine the most efficient means of diagnosis, prevention and treatment. Rapidity and accessibility also represent important objectives for new research relating to diagnostic tests. As shown in the current study, Ct values cannot reliably determine the viral load in the symptomatic patients. In the interim, stringent and well-applied measures of prevention and detection can aid in the prevention and spread of COVID-19.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

None.

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