



ROLE OF RT-PCR CT VALUE IN PREDICTING SEVERITY OF COVID-19 INFECTION: IS IT STILL REQUIRED???

Microbiology

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ABSTRACT

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by a newly discovered strain of coronavirus called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Patients are detected by gold standard test i.e. RT-PCR along with radiological and clinical investigations and thereby treated accordingly. This test enables early detection of viral genome in clinical samples and allows the health professionals to isolate the patient and prevent spread of the disease. Several studies have shown link between this viral load marker and severity of disease. Therefore we studied the correlation of the Ct value of RT PCR for COVID 19 with severity of disease admitted with COVID 19 at our hospital to find out whether it provides any valuable information to clinicians or not.

KEYWORDS

RT-PCR Ct value, disease severity, viral load, ICMR.

INTRODUCTION–

During Covid scenario, Real Time Reverse Transcription Polymerase Chain Reaction (Real Time RT-PCR) had become the gold standard test for detection of SARS-CoV-2. A positive test allows the health professionals to quickly isolate the patient and prevent spread of the disease. The Ct (cycle threshold) is defined as the number of cycles required for the fluorescent signal to cross the threshold (i.e. exceeds background level). This test provides an estimate of the viral load¹. The cycle threshold (Ct) values of the PCR reaction correlate inversely with the viral load.² It is being assumed by some researchers / clinicians that high viral load directly correlates with increased infectiousness and severity of disease.^{3,4} The cycle threshold (Ct) in RT-PCR SARS-CoV-2 tests has gained importance as a prognostic marker for severe disease in patients with COVID-19 illness. Several studies have been taken up showing link between this viral load marker and severity of disease. One review published in July 2020 in Infectious Diseases and Therapy reported an association between lower Ct values and not only worsening disease but also progression to severe disease and complications.⁵

Keeping in mind, the guidelines given by ICMR regarding Ct value⁶, it may depend on various factors like method of specimen collection, specimen source, transport, and the time from infection to collection to analysis. Matthew Binnicker, PhD, director of clinical virology at Mayo Clinic in Rochester, Minnesota, mentioned similar concerns in the Journal of Clinical Microbiology and stated that PCR Ct values may vary significantly between assays, even those using the same gene target.⁷ We therefore studied the correlation of the Ct value of RT PCR for COVID 19 with severity of disease admitted with COVID 19 at our hospital and whether or not they could provide valuable information to clinicians for more tailored decision-making.

MATERIALS AND METHODS-

A retrospective study was done on random 400 patients over a period of 2 months. i.e. 17th July till 17th September. Throat or deep nasal cavity swabs were collected in viral transport media (VTM) taking all the universal precautions. Samples were properly labelled, packed in triple packaging layer and were transported to laboratory maintaining an adequate cold chain. All the samples were processed in bio safety laboratory-3 by taking biosafety precautions. Their RNA was extracted by using Perkins Extraction Kit and their SARS-CoV-2 was diagnosed by RT PCR assay by using Quantipluss multiplex Covid-19 detection kit by Huwel Lifesciences Pvt. Ltd., Hyderabad, India which targeted 2 genes i.e. E gene(envelope protein) and N gene(nucleocapsid protein). (Table:1) The patient population was

segregated into those with severe disease non severe disease according to Covid Protocol given by Govt. Of India MOHFW.⁸

Table 1: Interpretation of results by Quantipluss kit

E gene (FAM)	N gene (Yakima Yellow)	Internal Control (Ct5)	Interpretation	Conclusion
✓	✓	✓	2019-nCoV RNA Detected	Proceed for further Analysis
-	✓	✓	2019-nCoV RNA Not Detected	
✓	-	✓	B-beta CoV specific RNA detected*	
-	-	-	Possible inhibition of PCR	Dilute the RNA sample (1:10) and repeat the Assay

RESULTS-

Out of 400 patients, there were 68% male and 32% females. We divided these patients into 4 age groups as 0-15 years, 16-40 years, 41-60 years and >61 years. We found that maximum patients belonged to age group of 16-40 years i.e. adults whereas least positivity was found in age group 0-15 years.

We also classified Ct values in 3 ranges i.e. 15-25, 26-30 and >31 and the severity of disease was correlated with the same. In terms of severity of disease maximum patients belonged to age group 40-60 years of age. Severity was assessed by following Covid Protocol given by Govt. Of India MOHFW⁸. Maximum patients had Ct value in between (26-30). There were total 126(31.5%) patients in whom the disease took a severe pace. Moreover there were few patients who were asymptomatic but showed positive test results, under cluster sampling and contact tracing. Even there were some patients who were immunocompromised having high Ct value.

Table 3 shows that there is no correlation of Ct values with severity of disease. Therefore rejecting the hypothesis that, lower Ct value is associated with a more severe disease; severity should not be correlated with RT-PCR Ct value itself as it may also depend on various other factors as well.

Table-2: Comparison of different parameters and ct values of patients with their age group.

Age	No. of patients (%)	No. of severe Patients (%)	Asymptomatic Patients	Patients under CT value (15-25)	Patients under Ct value (26-30)	Patients under CT value >31	Immunocompromised patients
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0-15 YR	24(6%)	6(4.8%)	4(5.7%)	12 (12.5%)	13(5.2%)	6(10.7%)	00
16-40 YR	282 (70.5%)	37 (29.4%)	59 (84.3%)	54 (56.3%)	175 (70.6%)	37 (66.1%)	9(15%)
41-60 YR	69 (17.25%)	79 (62.7%)	8(11.4%)	18 (18.7%)	49 (19.8%)	8(14.3%)	33 (55%)
>61Y R	25 (6.25%)	4(3.2%)	7(10%)	12 (12.5%)	11(4.4%)	5(8.9%)	18 (30%)
Total (N)	400 (100%)	126 (31.5%)	70 (17.5%)	96 (24%)	248 (62%)	56(14%)	60 (15%)

Table-3: Percentage of patients with different Ct values.

Interpretation	Ctvalue 15-25 (%)	CT value 26-30(%)	CT value >31(%)	Total
No. of patients (n=400)	96 (24%)	248(62%)	56(14%)	400
No. of severe	36 (28.6%)	74(58.7%)	16(12.7%)	126 (31.5%)
Patients (n=126) (p=0.126)				
No. of asymptomatic Patients (n=70) (p=0.330)	32(45.7%)	24(34.2%)	14(20%)	70 (17.5%)
Immunocompromised patients(n=60) (p=0.240)	17(24.3%)	21(30%)	22(36.7%)	60(15%)
Others (n=144)	11(7.6%)	129 (89.6%)	04(2.8%)	144(36%)

*Non significant: P>0.05

DISCUSSION:

In our study we could not demonstrate any correlation between Ct values and severity of disease. The Indian Council of Medical Research (ICMR) claimed that adding cycle threshold (Ct) values to coronavirus test reports is not necessary as the concept is scientifically flawed.

The ICMR also stated that patients in early symptomatic stages may show high Ct value, which could subsequently change, in such cases, the high value will give a false sense of security. Severity of Covid-19 depends on a host factors, besides the viral load. Some patients with low viral load may suffer from very severe disease due to triggering of the immunological response.

Samples from asymptomatic and mild cases show similar values to those who develop severe disease.

Hence we conducted a study in favour of this statement where total 400 COVID-19 patients were contacted and followed to assess their level of severity and compared with the cycle threshold values of RT-PCR results. 282 (70.5%) patients were found to be in the 16-40 years age group with least in 0-15 years(6%) which is correlated with the study done by Mehta N. S. Et al¹⁰ and their study says that children are do affected by COVID-19, but extant data suggest they are less likely to be affected than adults.

Maximum patients 248(62%) in our study were falling under CT value (26-30) which was in favour with the study done by N. Asai et al¹¹ who also observed that about 70 % patient showed Ct-values >25.

When we observed cycle threshold value of severely affected patients, We found 126 (31.5%) (n=400, P=0.126) patients to be affected severely. Out of which 74(58.7%) were having (26-30) Ct value which is correlated with the study done by Ade C. et al¹².

Out of 70 asymptomatic patients (n=400, P=0.330), there were 32(45.7%) patients in our study who were falling under Ct value(15-25) although they were asymptomatic as per study by Zhou R¹³ which cited that patients may be asymptomatic but they still have a certain period of viral shedding, which suggested the possibility of transmission during the asymptomatic period. Similarly studies have not found any difference between viral loads as determined by Ct values between symptomatic and asymptomatic patients.¹⁴

Also we observed, out of 60 (n=400, P=0.240) immunocompromised patients, 22(36.7%) patients, inspite of being immunocompromised were having Ctvalue (>31) which is very much in favour with the study

done by Choi B et al.¹⁵ This could possibly be attributed to much stricter application of social distancing procedures also that the patient was aware of that she might be at higher risk of severe COVID-19 infection.¹⁶

CONCLUSION:

CT value is not a definite criteria for assuming severity of disease. Labs should not routinely report the Ct value. Instead, they should provide it to physicians verbally on a case-by-case basis so that clinicians may better understand the dynamics of the disease. The Indian Council of Medical Research has also recommended the same.¹⁷ Better ways of estimating the viral load of COVID 19 and disease severity/infectivity are needed. Decisions about predicting severity of disease should be based on clinical parameters including age, comorbidities and laboratory parameters including the absolute lymphocyte count, C reactive protein levels and D- Dimer levels rather than the Ct value⁹.

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