

## Association of Cycle Threshold Values of rRT-PCR with Clinico-Demographic Profile of COVID-19 Patients in a Tertiary Care Hospital of Tripura, North East India

Gourab Chakraborty<sup>1</sup>, Anup Saha<sup>2</sup>, Pradip Kumar Das<sup>3</sup>, Debosmita Paul<sup>4\*</sup>, Niladri Sekhar Das<sup>5</sup>, Jayanta Debnath<sup>6</sup>, Sagnik Bhattacharjee<sup>7</sup>, Antara Roy<sup>8</sup>

<sup>1,2,3,4,5,6,7,8</sup>Department of Microbiology, Tripura Medical College & DR BRAM Teaching Hospital, Hapania- 799014. P.O- ONGC, Agartala, Tripura

Received: 25-06-2023 / Revised: 28-07-2023 / Accepted: 30-08-2023

Corresponding author: Dr. Debosmita Paul

Conflict of interest: Nil

### Abstract:

**Background and Objective:** At the end of 2019, SARS CoV 2 virus causing COVID-19 disease had emerged in whole world leading to the pandemic. Real-time reverse-transcriptase polymerase chain-reaction (rRT-PCR) of nasopharyngeal /oropharyngeal swab has been the gold standard test for detection of SARS-CoV 2 infection. Ct value of RT PCR can be used as indirect marker of viral RNA copy. Aim of the study was to elucidate the association between Ct value and clinic- demographic profile of COVID-19 patients.

**Methods:** A retrospective cohort study was conducted in the Department of Microbiology, Tripura Medical College & DR BRAM Teaching Hospital on 1471 COVID-19 patients who were diagnosed by rRT PCR during May 2021 to December 2022. Required data were collected from ICMR COVID-19 portal and Sample referral form(SRF) and subsequently analysed from May to June 2023. Age group and gender were taken as demographic variables. Ct values were categorized as group A(10-19),group B(20 -28) and group C(29-36) to establish their relationship with clinical symptoms and demographic characters. For statistical analysis Chi square test was utilized and p-value <0.05 was considered significant.

**Results:** Out of 1471 rRT PCR confirmed COVID-19 patients highest cases were found among 21 to 40 years age group, 524(35.62%) and male patients,927(63.02%). Among symptomatic patients, 698 (47.45%) commonest complaint was fever, 453 (64.89%) followed by cough 341(48.85%) and breathlessness 174(24.92%). In relation to Ct value symptomatic patients, 149 (53.40%) were more in group A but there were more asymptomatic patients, 434(52.86%) and 209(56.33%) in group B and C respectively with significant association (P =0.0001).Ct value and clinical symptoms were also significantly associated (P=0.0000) in which fever and cough were more with Ct group B &C but breathlessness were mostly in Ct group A.

**Conclusion:** Young age group with male preponderance were observed among COVID-19 patients of whom asymptomatics were more than symptomatics. Ct values were significantly related with symptomatology where fever and cough were largely associated with higher Ct value and Breathlessness was with lower Ct value. Therefore lower the Ct higher the viral load resulting in severe COVID-19 disease and vice versa.

**Keywords:** Breathlessness, Corona Virus Disease-19, Fever, Real-Time Reverse Transcriptase Polymerase Chain Reaction.

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### Introduction

Severe acute respiratory syndrome corona virus 2 (SARS-CoV2) causing Corona virus disease 2019 (COVID 19) has emerged and led to worldwide health crises resulted in global pandemic.[1] The World Health Organization declared a global pandemic on March 11, 2020. [2]

COVID-19 was first reported in December 2019 in Wuhan, China while in India first case was identified on January 30, 2020.[3,4] Our state Tripura is situated in North-eastern region of India

where first COVID-19 patient was diagnosed on 7 th April 2020.[5]

The mode of transmission of SARS-COV-2 is inhalation of droplets and touching fomites contaminated with the virus.[6] The SARS COV-2 virus infects people of all ages with evidences of two groups of people who are at higher risk for developing COVID-19. One group are the elderly people and the other group are the adolescent and young adults. [7,8]

COVID-19 results in asymptomatic, mild, or severe respiratory tract infections in human beings.[9] The clinical course of COVID-19 evolves in at least three phases, the first phase with cough, fever, wheezing, fatigue, headache, diarrhea and dyspnea. The second phase with the rapid appearance of bilateral pneumonia infiltrates with variable degree of hypoxemia and third phase in which some patients develop respiratory failure leading to death.[10]

Real-time reverse-transcriptase polymerase chain-reaction (rRT-PCR) of nasopharyngeal/oropharyngeal swab has been the gold standard test for detection of SARS-CoV 2 infection.[11] There are many viral targets to detect the virus including nucleocapsid (N), open reading frame (ORF) 1a, RNA dependent RNA polymerase (RdRp), spike (S), envelope (E).[12] COVID-19 testing is designed as a two target system, where E gene detects universal SARS corona viruses and a confirmatory testing with a second set of primers(using ORF 1ab or RdRp gene) which detects only SARS-CoV2.[11]

The cycle threshold (Ct) values of RT PCR represent the number of amplification cycles that needed by the target gene to surpass a certain threshold level. Ct values are inversely proportional to viral load and can be used as an indirect way of quantifying viral RNA copy numbers.[13] The amount of virus exposure at the beginning of infection can increase the severity of the disease that is related to a higher viral load. Viral load in COVID-19 patients might be associated with disease infectivity, disease phenotype, morbidity and also mortality.[14,15] It has been assumed that low Ct value is related to high viral load which results in more clinical manifestations of SARS COV 2 infection or COVID-19 disease and vice versa. Moreover there is very limited research and publications related to clinical utility of Ct values for aiding in the prognostication of patients with COVID-19 in the state of Tripura. Therefore this study was undertaken with an aim to determine the association between Ct values of rRT PCR, clinical features & demographic profile of COVID-19 patients.

### Study Objectives

- To assess the proportion of rRT PCR positive COVID-19 patients with respect to clinico-demographic profile and Ct values.
- To evaluate the association between rRT PCR Ct values and clinico-demographic profile of COVID-19 patients.

### Materials & Methods

This is a retrospective cohort study which was conducted at Microbiology Department of Tripura Medical College & DR. BRAM Teaching Hospital, Tripura. This study proposal was approved by

Institutional ethical committee with reference no IEC/SFTMC/2023/3/002. A total of 1471 COVID-19 patients were taken as sample size for this study. All suspected COVID-19 patients of all age group and gender visited our hospital seeking medical advice during the period from May 2021 to December 2022 were accounted the target population of this study. After filling up of ICMR approved sample referral form (SRF) nasopharyngeal swabs/oropharyngeal swabs were collected from each suspected patient as per standard protocol [16]. Samples were processed at NABL accredited Virology service laboratory (VSL) of Microbiology department which is also ICMR approved for SARS CoV 2 rRT PCR test.

Viral RNA extraction was done according to Viral RNA Extraction Miniprep Kit protocol manufactured by mdi membrane technologies.[17] rRT-PCR was performed by the ICMR approved DiAGSure™ n CoV 19 Detection Assay(Multiplex, TaqMan based) protocol developed by GCC BIOTECH. This assay protocol was designed targeting 'ORF 1ab' (confirmatory) and 'E' gene along with internal control(IC) RNase P, Positive and Negative control. As per kit literature Ct value cut-off for ORF 1ab and E gene was  $\leq 36$  and for IC it was  $\leq 35$ . Results of the rRT PCR were interpreted as follows:

**Positive** if 'ORF1ab' gene positive, 'E' gene positive/negative, and IC positive.

**Negative** if 'ORF 1ab' gene negative, 'E' gene positive/negative and IC positive.

**Invalid** if ORF 1ab negative, E negative and IC negative. [18]

**Inclusion criteria:** Among all targeted population who were rRT PCR positive for SARS CoV 2 RNA.

**Exclusion criteria:** Patients whose rRT PCR test result were negative or invalid for SARS CoV 2 RNA.

All required data including patients age and sex ,whether symptomatic or asymptomatic and Ct values of 'ORF 1ab' which is confirmatory gene were collected from ICMR COVID-19 Portal Presenting complaints of all symptomatic patients such as Fever, cough, sore throat, breathlessness, loss of taste, loss of smell, diarrhoea were retrieved from ICMR approved SRF.

Age group and gender were considered for demographic factors. For better analysis of variables in relation to viral load, the Ct values of 'ORF 1ab' gene were categorized as Group A: 10-19 (High viral load), Group B: 20-28 (Medium viral load) and Group C: 29-36 (Low viral load).

Collected data was entered in Microsoft excel 2007 and analyzed using SPSS version 20. All results were expressed in frequency and percentage. Chi

square test was applied to find out the association between Ct values and clinico-demographic factors. A 'p' value of < 0.05 was considered significant.

**Results**

In this study we enrolled 1471 r RT PCR confirmed COVID-19 patients and evaluated in respect of different variables i.e age-group, gender, clinical profile and Ct value range to explore the relationship between clinico-demographic features and Ct value. Age group wise highest no of patients

524(35.62%) were found in 21 to 40 years followed by 445(30.25%) in 41 to 60 years, 354(24.07%) in 61 to 80years, 89(6.05%) in less than 20 years and 59(4.01%) in 81 or above age group. Of the total 1471, Male and Female patients were 927(63.02%) and 544 (36.98%) respectively.[Table 1]At the time of sample collection 773 (52.54%) patients were asymptomatic but 698(47.45%) patients were presented with one or more symptoms such as fever, cough ,breathlessness, loss of taste, loss of smell, sore throat and diarrhea.[Table 1 and 2]

**Table 1: Distribution of COVID-19 patients with respect to different variables**

Variables	Total(n= 1471)	
Age group	Less than 20 years	89 (6.05%)
	21 to 40	524 (35.62%)
	41 to 60	445 (30.25%)
	61 to 80	354 (24.07%)
	81 and above	59 (4.01%)
Gender	Male	927 (63.02%)
	Female	544 (36.98%)
Symptomatic	Yes	698 (47.45%)
	No	773 (52.54%)
Ct value	Group A(10 -19)	279 (18.96%)
	Group B(20 -28)	821 (55.81%)
	Group C(29 -36)	371(25.22%)

The distribution of patients in three Ct group were as follows: 821 (55.81%) patients in Ct group B (20 to 28), 371(25.22%) in group C(29 to 36) and 279 (18.96%) in group A(10 to 19).[Table 1] Among symptomatic patients(N=698) most common presenting complaints were fever, 453(64.89%) followed by cough, 341 (48.85%), Breathlessness,174 (24.92%), loss of smell, 111 (15.90%),loss of taste,84 (12.03%) ,sore throat,70 (10.02%), diarrhea, 34 (4.87%) and others 32(4.58%)[Table 2]

**Table 2: Presenting complaints of symptomatic COVID-19 patients (N= 698)**

Symptomatology	Total(n= 698)
Fever	453 (64.89%)
Cough	341 (48.85%)
Sore throat	70 (10.02%)
Breathlessness	174 (24.92%)
Loss of taste	84 (12.03%)
Loss of smell	111 (15.90%)
Diarrhea	34 (4.87%)
Others	32 (4.58%)

Patients of different age group were analyzed against three Ct group by chi square test and p value was found to be 0.922 which is statistically insignificant. In all three Ct group, patients of 21 to 40 years age group were maximum and found to be 100 (35.84%) in group A, 294 (35.80%) in group B

and130 (35.04%) in group C.[Table 3]. The difference in Male and female patients among Ct groups were significant at p value 0.0001. Male patients were predominating with 193(69.17%) in group A, 479(58.34%) in group B and 255 (68.73%) in group C.[Table 4]

**Table 3: Association of Ct value and age group distribution**

Age group	Ct value group			P value
	Group A(10-19) N=279	Group B(20-28) N=821	Group C(29 – 36) N=371	
Less than 20 years	21 (7.52%)	44 (5.35%)	24 (6.46%)	<b>0.922</b>
21 to 40	100 (35.84%)	294 (35.80%)	130 (35.04%)	
41 to 60	86(30.82%)	244 (29.71%)	115 (30.99%)	
61 to 80	63(22.58%)	205 (24.96%)	86 (23.18%)	
81 and above	09(3.22%)	34 (4.14%)	16 (4.31%)	

#Chi square test, p value < 0.05 taken as a statistically significant

**Table 4: Gender distribution in respect of Ct value group with p value**

Gender	Ct value group			P value
	Group A(10-19) N=279	Group B(20-28) N=821	Group C(29 – 36) N=371	
Male	193(69.17%)	479(58.34%)	255 (68.73%)	<b>0.0001</b>
Female	86 (30.82%)	342(41.65%)	116 (31.26%)	

#Chi square test, p value < 0.05 taken as a statistically significant.

Table 5 showed symptomatic patients 149(53.40%) were greater in group A Ct value but among patients of group B and group C Ct asymptomatics were higher with 434(52.86%) and 209(56.33%) respectively.

Their difference is statistically significant with P value 0.0001. Analyzing symptomatology with Ct value, fever 242(53.42%), cough 213(62.46%) sore

throat 42(60.00%) loss of taste 43( 51.19%) and loss of smell 51(45.94%) were mostly associated with Ct range 20 to 28.

Whereas Breathlessness 98(56.32%) were more in Ct range 10 to19.The association between symptoms and Ct group were found to be significant (p=0.0000)[Table 6]

**Table 5: Clinical profile of patients in respect of Ct group with p value**

Clinical profile	Ct value group			P value
	Group A(10-19) N=279	Group B(20-28) N=821	Group C(29 – 36) N=371	
Symptomatic	149(53.40%)	387(47.13%)	162(43.66%)	<b>0.0001</b>
Asymptomatic	130(46.59%)	434(52.86%)	209(56.33%)	

#Chi square test, p value < 0.05 taken as a statistically significant

**Table 6: Symptomatology against Ct group with p value**

Symptomatology	Ct value group			P value
	Group A(10-19) N=279	GroupB(20-28) N=821	Group C(29 – 36) N=371	
Fever(N=453)	112(24.72%)	242(53.42%)	99 (21.85%)	<b>0.0000</b>
Cough(N=341)	87(25.51%)	213(62.46%)	41(12.02%)	
Sore throat(N=70)	21(30.00%)	42(60.00%)	07(10.00%)	
Breathlessness(N=174)	98(56.32%)	54(31.03%)	22(12.64%)	
Loss of taste (N=84)	24(28.57%)	43(51.19%)	17(20.23%)	
Loss of smell(N=111)	34(30.63%)	51(45.94%)	26(23.42%)	
Diarrhea(N=34)	8(23.52%)	21(67.74%)	5(14.70%)	
Others(N=32)	9(28.12%)	18(56.25%)	5(15.62%)	

#Chi square test, p value < 0.05 taken as a statistically significant

**Discussion**

In this retrospective cohort study clinico demographic features of COVID-19 patients were analyzed against three group of Ct range of rRT PCR to understand the impact of Ct value on the disease severity. All total 1471 COVID-19 patients included in this present study were diagnosed by rRTPCR at VSL of Tripura Medical College and DR BRAM Teaching Hospital during the period of May 2021 to December 2022 and subsequently data analysis was done in the month of May to June 2023. At the beginning demographic features of patients revealed that age group 21 to 40 years (35.62%) were found to be largely affected followed by 41 to 60 years (24.07%)and least number of patients were found in 81 or above age group (4.01%). In addition among all study subjects males (63.02%) were more than females (36.98%).To compare with other studies done by Jamil et al[3] and Mehrotra A[19] which also showed highest no of patients in 21 to 30 years

(32.78%) and 21 to 40 years(48.6%) of age respectively. Regarding gender distribution similar findings were also observed by Farhana et al(63.5% vs 36.5%).[20]

A statistically significant (p=0.0001) association was observed between Ct value group and gender where male patients were maximum in all Ct group which is not similar according to study done by Sadhegi et al (P=0.48).[21] On the other hand our study showed no significant difference (p=0.922) in Ct values among different age group of patients which is not concordant with the observation of Sadhegi et al(P=<0.001).[21] Here it is to be mentioned that all the patients included were diagnosed during second and third wave of pandemic though demographic features varied according to the strain of virus circulated and geographical location. Further in this geographical area, males being an active and working member of the family are involved most of the outdoor

activities thereby exposing themselves to the virus causing more susceptibility to infection.

Our study showed more asymptomatic COVID-19 patients (52.54%) than symptomatics (47.45%) which is similar to previous study done by Jamil et al.[3] This observation might be explicated in view of highest distribution of COVID-19 patients among immunocompetent young aged population. Among symptomatic patients most common presenting complaints were fever (64.89%) followed by cough (48.85%) and breathlessness (24.92%). This findings are also concordant with studies done by Husaini et al [22] and soni et al.[23]

In this study we found highest proportion of patients 55.81% in the Ct range of 20 to 28 (group B) which is in the line of observations made by Sadhegi et al[21] where 75.7% patients had a Ct between 21 to 30. According to Dres et al (2021) Ct values of rRT PCR were used as the indicators of RNA viral load in the samples, the lower the Ct, higher the viral load.[24] We observed among patients with lower Ct value (10 to 19) symptomatics (53.40%) were higher than asymptomatics. Contrary to that asymptomatic patients (56.33%) were more than symptomatics in higher Ct group (29 to 36). There was a significant association ( $P=0.0001$ ) between clinical profile and Ct value. Here we conclude that lower the Ct, higher the viral load resulting in more viral spread and illness.

Majority of patients with fever, cough, loss of smell were related to medium to higher Ct range. Another interesting finding in our study was breathlessness (56.32%) being mostly associated with lower Ct range of 10 to 19 which were relating to high viral load indicating severe COVID-19 disease. However various factors could affect the Ct values including sample collection technique, type of specimen, sampling time and viral kinetics. In addition to that different reagent kits, pipetting defects, type of RT PCR also influenced the Ct value to some extent.[25]

**Limitations:** One limitation of this study is lack of follow up of all participating patients including symptomatics and asymptomatics. As a result their outcome i.e whether cured and discharged or succumbed to the disease were also not known, which would have given us better understanding of the impact of Ct value on disease morbidity and mortality. Besides this present study could not include the patients of first wave of pandemic and because of hospital based study demographic characteristics of COVID-19 patients were also not determined properly. Therefore it should be integral to conduct community based study during such type of pandemic to elucidate the exact socio demographic features of the patients. Additionally,

we can further carry out the whole genome sequencing of RNA extracts of COVID-19 patients to acquire the knowledge of variants transmitted during different phase of pandemic which might help us to formulate preventive measures and in vaccine development as well.

### Conclusion

Ct value of rRT PCR could be used as an indicator of Viral RNA load in COVID-19 patients. That means lower Ct value corresponding to the higher viral load which results in severe COVID-19 disease and vice versa. However various preanalytical, analytical and post analytical factors are there which could influence the Ct value of rRT PCR. In our study, symptomatics were more among patients with lower Ct and asymptomatics were more among patients with higher Ct. Except Breathlessness all other symptoms such as fever, cough, sore throat, loss of smell, loss of taste were related to a bit higher Ct value. But most patients with breathlessness were belonged to lower Ct value. Moreover it was also proved statistically that there is a significant association between Ct value and clinical symptoms of COVID-19 patients.

### Acknowledgement

We acknowledge Indian council of medical research (ICMR) for kind approval to start RT PCR test for COVID-19 diagnosis at VSL, Microbiology department of Tripura Medical College, Agartala, Tripura. We thank department of health and family welfare, Govt. of Tripura and our hospital management for supplying equipments, reagents and other logistics to deliver uninterrupted patient service. Lastly we must acknowledge hard work and dedication of our health care workers who were involved in sample collection, handling and processing of sample and report generation.

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