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Original Research Paper

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ROLE OF CYCLE THRESHOLD VALUES OF RTPCR AND COMPUTED TOMOGRAPHY SEVERITY SCORE IN COVID 19 PANDEMIC

Dr Yoganand Patil	MD Pathology. DNB Faculty, Head of Pathology Department
Dr Mrunal Kesari*	MD,DNB Pathology. DNB Faculty, Pathologist. *Corresponding Author
Dr Harshad Kesari	MD, DNB Pharmacology, Lecturer, KEM Hospital Mumbai.
Dr Shweta Agrawal	MD Pathology, Senior Resident.
Dr Priyanka Gadge	MBBS, Junior resident, Pathology.
Dr Hafeezunissa Rehman	Medical Director, Jagjivanram Hospital.

ABSTRACT

Background: The WHO declared the outbreak of corona virus disease as a public health emergency of international concern on 30 January 2020. Real Time RT-PCR is the gold standard test for detection of SARS-CoV-2. Chest CT has been proposed as an ancillary approach for screening individuals with suspected COVID-19 pneumonia, especially RTPCR false negative cases, during the pandemic period.

Methods: This study was a retrospective study conducted at a tertiary care center in Mumbai, India. This study included all SARS-CoV2- RNA RTPCR positive patients (n=681) from February to April 2021. RTPCR was performed and Ct value was analyzed. Chest CT severity score (CSS) was calculated and correlation with disease severity was analyzed using Spearman's rho nonparametric test.

Results: Out of 681 patients, 36.7% were asymptomatic and 63.3% were symptomatic. HRCT scan was done by 60 patients (38 hospitalized and 22 home isolated patients). Mean CSS in mild, moderate, and severe cases was 3.9, 10.5 and 18.5 respectively. Mean Ct value in mild, moderate and in severe cases was 22, 22.5 and 24.1 respectively. Significant correlation was found between CSS and the disease severity (p value << 0.01). While no correlation found between CT Value and disease severity (p value = 0.658)".

Conclusion: RT-PCR assay plays a vital role in early diagnosis and early isolation for individual patients. Chest CT severity score correlates with disease severity, identifies patients at risk of progression, and ensures better treatment outcomes and optimal utilization of medical resources.

KEYWORDS : COVID-19, Computed tomography severity score, Cycle threshold value, Disease severity

INTRODUCTION:

The World Health Organization (WHO) declared the outbreak of novel corona virus disease (COVID-19) as a public health emergency of international concern on 30 January 2020.[1] Real Time Reverse Transcription Polymerase Chain Reaction (Real Time RT-PCR) is the gold standard test for detection of SARS-CoV-2 (Severe acute respiratory syndrome coronavirus-2) Chest CT (Computed tomography) has been proposed as an ancillary approach for screening individuals with suspected COVID-19 pneumonia, especially RTPCR false negative results during the pandemic period.[3]

This study discusses the relevance and utility of cycle threshold(Ct) value of RTPCR test and CT severity score in Covid-19 patients according to disease severity.In this pandemic, better understanding of interpretation of Cycle threshold (Ct) value and chest CT findings are important for early identification, isolation, contact tracing, and rationalization of infection control measures, risk stratification, and management of cases.

AIMS:

1. To correlate the disease severity with RTPCR Ct value.
2. To correlate the disease severity with CT severity score (CSS).

MATERIALS AND METHODS:

Patients and data collection: This study was a retrospective study conducted at a tertiary care center in Mumbai, India. This study included all SARS-CoV2 RNA RTPCR positive patients (n=948) from February to April 2021. Out of these 267 patients were excluded who have not responded to the phone calls for survey. After exclusion of these patients, 681 patients were available for analysis. Covid management was as per ICMR (Indian council of medical research) protocol.

Data was collected from Hospital records and LIS in hospitalized patients. A telephonic survey was done to collect additional data about dates of vaccination of first and second dose, previous history of Covid infection in home quarantined cases. Spearman's rho nonparametric test was calculated to find association between disease severity and CT severity score and Ct (cycle threshold) value of RTPCR. Informed consent was obtained from all the cases involved in the study.

Diagnosis of COVID-19 infection: In our study we stratified patients into mild moderate and severe cases according to MoHFW (Ministry of Health & Family Welfare) guidelines. Mild cases are defined as a patient with Upper respiratory tract symptoms (&/or fever) without shortness of breath or hypoxia, moderate case defined as a patient with Respiratory rate > 24/min, breathlessness and SpO2: 90% to < 93% on room air and severe case as a patient with Respiratory rate >30/min, breathlessness and SpO2 < 90% on room air.[4]

Viral RNA was extracted by using the KingFisher™ Flex Magnetic Particle Processor with 96 deep well Head and the MagMAX™ Viral/Pathogen Nucleic acid isolation kit, and RTPCR was performed using Pathodetect kit which targets the following three SARS CoV2 specific genes: RNA- dependent RNA polymerase (RdRp), envelope (E gene) and nucleocapsid (N gene). In our study E gene was considered for the Ct value.

A positive and negative control was included into each run to generate a valid result. A Ct value of <37 was defined as a positive result. Viral load was categorized as high (<24), medium (24-35) and low (>35).

HRCT imaging evaluation: CT chest was indicated in patients who were hospitalized and had dyspnea and some

comorbidities. Some mild cases in our study had done CT chest outside hospital because of anxiety. Image analysis was performed on 64 slice and documented in lung and mediastinal windows using additional thin 1mm sections using a high resolution algorithm at the interval of 10 mm sections. CT severity score (CSS) was calculated by quantifying the disease affected areas for each lobe (The total score is the sum of approximate percentage of involvement of each lobe of the five lobes of lung by disease - 0% = 0 point, Less than 5% = 1 point, 5-25 % = 2 points, 25-50 % = 3 points, 50-75 % = 4 points, 75-100% = 5 points, Max. 25 points.

CT severity Score: <8- Mild, 9-15 – Moderate, More than 15 – Severe.

Statistical analysis: Descriptive statistics were presented as percentage and mean (average) in data. Categorical variables (CSS and Ct value) were compared using the Spearman's rho nonparametric correlation test.

RESULTS:

Out of 681 patients, 36.7% (250/681) were asymptomatic and 63.3% (431/681) were symptomatic (table 1). Mean age of mild, moderate and severe cases was 42.7, 52.3 and 62.3 years respectively. Fever was the most common symptom encountered in patients (320/431), followed by dyspnea (28/431) and Loss of smell & taste (26 /431); summarized in Table 2. Among all patients 35.6% (243/681) were hospitalized and 60.7% of them hospitalized for <7days, 39.3% were admitted for >7days and 7 of them died during hospitalization (table 3). Remaining patients (438/ 681) were advised for home isolation as they were asymptomatic and some had mild symptoms. According to ICMR guidelines, patients were categorized into mild, moderate and severe cases. Comorbidities were seen in 16% (109/681) of cases and hypertension and diabetes were the commonly presented co-morbidity (table 4). HRCT was done in 60 patients (38 hospitalized and 22 home isolated patients). Of these 56.7% (34/60) cases had mild CSS (<8), 13.3% (8/60) had moderate CSS (8-15) and 30% cases (18/60) had severe CSS (>15) (table 5).

Mean CSS in mild cases was 3.9, in moderate cases was 10.5 and in severe cases was 18.5 (table 6, chart 1). Mean Ct value (viral load) in mild cases was 22, in moderate cases was 22.5 and in severe cases 24.1 (table 6, chart 2).

A strong positive correlation was found between the CT Scan Score and the disease severity (Spearman's rho = 0.910, p << 0.01). Whereas correlation between CT Value for the E-gene and disease severity was insignificant (Spearman's rho = 0.058, p = 0.658).

Vaccination was received by 13.9% (94/681), among them 80.9% (76/94) received only a single dose of Covid vaccine and 19.1% (18/94) received complete doses of vaccine (table 7).

Reinfection with corona virus (previous H/O Covid) present in 1.6% (11/681) cases.

Mortality rate among hospitalized patients was 2.8% (7/243); older age CT severity and comorbidities were the significant risk factors.

Table 1: Symptom status of the cases

Total	681	Percentage (%)
Asymptomatic	250	36.7%
Symptomatic	431	63.3%

Table 2: Clinical characteristics of the cases

	Mild	Moderate	Severe
Mean Age (years)	42.7	52.3	62.3
Fever (320/431)	287	18	15
Dyspnea (28/431)	17	3	8
Loss of smell & taste (26 /431)	24	2	0

Table 3: Duration of hospital stay in cases

Hospitalization	243/681	Percentage (%)
<7 days	148	60.7%
>7 days	95	39.3%

Table 4: Types of comorbidities in cases

Comorbidities	(109/681)
Diabetes mellitus	56
Hypertension	63
Others	24

Table 5: Number of cases according to CSS

CT severity score	Total (60/681)	Percentage (%)
Mild (<8)	34	56.7%
Moderate(9-15)	8	13.3%
Severe(>15)	18	30%

Table 6: Average CSS and Ct value in disease severity categories

Disease severity	Total (60)	CT severity score (Mean)	Ct value(Viral load) Mean
Mild	29	3.9	22
Moderate	13	10.5	22.5
Severe	18	18.5	24.1

Table 7: Vaccination status of cases

Vaccination		
No	Yes	
587 (86.1%)	94 (13.9%)	
	1st dose	2nd dose
	76 (80.9%)	18 (19.1%)

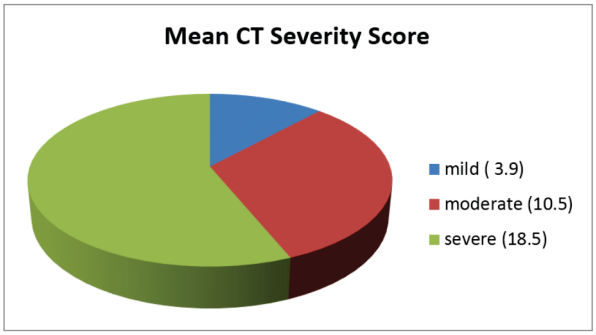

Chart 1: Mean (average) CT severity score in Covid 19 patients:

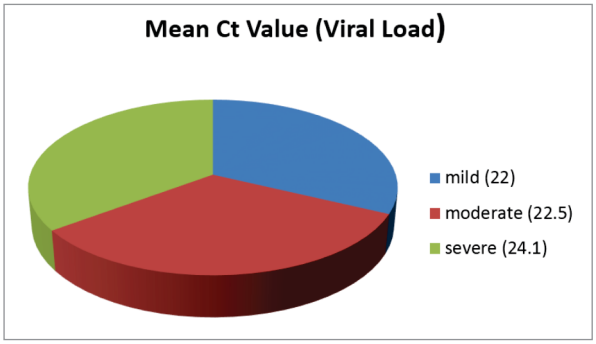

Chart 2: Mean (average) Ct value (viral load) in Covid 19 patients:

DISCUSSION:

Covid pandemic continues to be more challenging as newer SARS-CoV2 variants emerge. Corona viruses have structural proteins, including envelope (E), nucleocapsid (N), and spike (S) proteins. The ORF1ab, E, RdRp, N, and S genes are the targets used for the detection of SARS-CoV-2 by RT-PCR.CoV-2

is different from other known viruses due to multiple mutations on the sites of nonstructural proteins (NSP) 2 and 3, and the varying nature of virulence between different persons.[5]The variant viruses have increased infectivity, modest decrease in neutralizing activity, and may impact vaccine effectiveness via escape from vaccine-induced immunity, specifically by mutations in the spike protein.[6]

Clinical knowledge, pathogenesis pathways of COVID-19 are constantly evolving. According to Hasan K Siddiqi, there are three stages of Covid 19 infection with increasing disease severity. Stage I is the early infection or viral response phase during which symptoms of upper respiratory tract infection dominate. Stage II is the pulmonary phase when the patients develop full-blown pneumonia with all its associated symptoms and Stage III is the hyperinflammation phase when patients develop acute respiratory distress syndrome (ARDS), sepsis and kidney and other organ failures.[7] Most COVID-19 patients have a mild clinical course. Some cases show rapid deterioration from the onset of symptoms into severe illness with or without acute respiratory distress syndrome (ARDS).[8,9] Happy' or silent hypoxemia is a peculiar phenomenon in this disease in which inspite of pronounced arterial hypoxemia patients don't have proportional signs of respiratory distress. The respiratory center thus does not sense an uncomfortable sensation of breathing in early stages of disease as the lung mechanics are well-preserved. However, sudden and rapid respiratory decompensation may occur.[10]These patients have poor survival and need intensive medical resource utilization.

Constant evaluation of available evidence is essential to guide clinical suspicion, diagnosis, management, and mitigation of transmission of COVID-19. [11]Multiple laboratory parameters like hematology, biochemistry, molecular assays and imaging studies have been shown to have the diagnostic and prognostic capacity.

The primary and preferred method for diagnosis is collection of upper respiratory samples via nasopharyngeal and oropharyngeal swabs, and detection of SARSCoV-2 RNA by RT-PCR.[1]The cycle threshold or Ct value of a RT-PCR reaction is defined as the number of cycles at which fluorescence of the PCR product is detectable over and above the background signal. Theoretically, the Ct value is inversely proportional to the amount of genetic material (RNA) in the starting sample and lower Ct values generally correlate with high viral load.[12] It guides infection control, public health and occupational health decisions.[9]Efficacy of RT- PCR in the diagnosis of COVID19 infection is greatly dependent on the pre-analytical phase, including patient selection and material collection, and the extraction method of RNA and performance of RT-PCR test kit. It is being assumed by some researchers / clinicians that high viral load directly correlates with increased infectiousness and severity of disease.[9,13]

In our study mean Ct value in mild and moderate cases were approximately equal (22 and 22.5 respectively) and in severe cases slightly higher (24.1). So there was no significant correlation between Ct value and disease severity in our study (p value 0.658). Our results were concordant with the studies of Shah S et al[14], Arons et al [15], He et al[16]and discordant with the results of Huang et al[17], Liu et al[18] and Yu et al[19].

Severity of COVID-19 disease largely depends on host factors such as presence of pre-existing morbidities, immunological responses, drug history, contacts history and viral load.[20,21] Ct values may give a rough estimate of viral load. Some patients with low viral load have very severe disease. Specialized standards are required for quantitative assays like viral culture .These are currently unavailable for clinical use .Viral shedding persists for varying periods after symptoms' onset. Factors independently associated with prolonged respiratory viral shedding include fever (> 38.5°C), severe disease, old age (> 60), male gender, concomitant hypertension, steroid use, invasive ventilation, ICU admission, and lack of antiviral drugs.[22,23] Salvatore PP et al found that Ct values correlated with time elapsed since symptom onset . Ct values were significantly higher among asymptomatic cases and those presenting after 7 days of disease onset while lower in those with respiratory symptoms and within 7 days of disease onset.[24]Hence Ct value has no role in treatment plan of Covid patient. RT-PCR is associated with a relatively high number of false negative results. This can lead to an undetected transmission in either the community or within an institutional setting.[25]

A non-contrast high-resolution CT chest imaging plays an essential role in the early disease detection, particularly in patients with false-negative RT-PCR results.[12] In this study CSS correlated with disease severity (p value << 0.01). In severe cases CSS was higher (>15) our results were concordant with the study of Yagci A K et al[12] and Pan F et al.[4]

According to Li X et al study ground glass opacity (GGOs) on CT scan represents the acute-phase diffuse alveolar damage with airspace edema, bronchiolar fibrin, and interstitial thickening seen in early phases of the disease.[26]Late disease progresses with an intense production of pro-inflammatory cytokines that may trigger an uncontrolled autoimmune reaction. These findings may explain the higher prevalence of crazy-paving pattern and consolidation areas in late stages of disease.[27]A normal chest CT does not mean a person does not have COVID-19 infection - and an abnormal CT is not specific for COVID-19 diagnosis. The early/initial stage (0-4 days) can have a normal CT chest appearance.[28] Hence chest CT scan should be done at or after day 5 of symptom onset. CT scoring could help to stratify patient's risk and predict short-term outcome of patients with COVID-19 pneumonia. The extent of CT damage is highly correlated with various parameters of disease, including clinical staging and laboratory parameters.[29] Viral load of SARS CoV-2 in nasopharyngeal swab specimens is high in the early phases of COVID-19; it is not necessarily related to changes in chest CT. [12]

CT should be used sparingly and reserved for hospitalized, symptomatic patients with specific clinical indications for CT.[28]Ct scan is not screening test or a first-line test to diagnose COVID-19.Particular attention should be paid to the relationship between the results of testing, clinical conditions, time from symptom onset, the characteristics of the source patients, specimens and testing methods.[30]

Limitations:

Duration between RTPCR testing and symptom onset not recorded and was not fixed. Every CT scan was not done on a fixed interval after symptom onset. CT scan was done before admission, not according to ICMR criteria. Vaccination affecting immunopathogenesis as a confounding factor is not considered.

CONCLUSION:

Clinical course of Covid disease is unpredictable, ranging from asymptomatic to critical disease with multiorgan failure. RT-PCR assay plays a vital role in early diagnosis and isolation for individual patients. However Cycle threshold values of RTPCR do not correlate with disease severity. Chest CT should be used in suspected COVID-19 cases having negative RTPCR. Ideally chest CT scan should be done at day 5 of symptom onset. CT severity score correlates with disease severity, identifies patients at risk of progression, and ensures better treatment outcomes and optimal utilization of medical resources.

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