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COVID-19 INSIGHTS: ASSOCIATING CYCLE THRESHOLD WITH DEMOGRAPHY, CO-MORBIDITIES AND HEMATOLOGICAL PARAMETERS

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Abstract

Aim: COVID-19 is a multifactorial complicated disease which affects multiple organ systems and causes damage that may become severe and long lasting. The current study was conducted to evaluate the association between Cycle Threshold values for RT-PCR COVID-19 and demographic characters along with various co-morbidities including diabetes, hypertension, respiratory and cardiac illness. The correlation between Ct values and hematological parameters was also assessed to determine whether they could be suitable indicators for prognosis and management of patients. **Materials and Methods**: The study included 212 RT-PCR positive COVID-19 patients, demographic and laboratory parameters of whom were recorded and analyzed using two tailed test of significance and Karl Pearson's coefficient of correlation. **Results:** No significant association was found between demographic profile (age, gender), co-morbidities and Ct values. The association between haematological markers and Ct values also came out to be non-significant except for IL-6, where it was significant, indicating the direct relation between viral load and cytokine storm.

Keywords: Ct Value, COVID-19, Co-Morbidities, Hematological Parameters, Demographic Profile.

INTRODUCTION

Patients with the severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) exhibit varying degrees of illness severity, from lack of symptoms to severe symptoms of coronavirus disease (COVID 2019) [1, 2]. COVID-19 is a novel viral disease that, World Health Organisation (WHO) had declared a worldwide public health emergency. The virus is a member of the Coronaviridae family, B lineage of beta coronaviruses [3,4]. Older age, sex, and various comorbidities such as diabetes, obesity, hypertension, and cardiac disease are currently known factors which put the host at risk for the advancement of serious COVID infections. The levels of Interleukin-6 (IL-6), C-reactive protein (CRP), ferritin, D-dimer, and peripheral blood lymphocyte count have also been linked to the severity of the disease [2, 5]. Real-time reverse transcriptase polymerase chain reaction (RT-PCR) is an accepted molecular technique for diagnosis of COVID-19. The cycle threshold (Ct) value is defined as the number of amplification cycles required by the desired gene to be amplified beyond a threshold level. As a result, this value is inverse of the viral load and can be used to

determine the number of viral RNA copies present in a sample. Depending on the patients' burden of co-morbidities, it was also discovered that Ct values were related to the intensity of the disease. [1, 5]. The objective of the current research was to evaluate the relation between viral Ct values obtained from real-time RT-PCR and haematological markers as well as co-morbidities and demographic parameters.

MATERIALS AND METHODS

Study Design & Participants

It was a cross-sectional study conducted at Sharda Hospital, Greater Noida, from January 2021 to June 2022. The hospital was requisitioned as a level-3 centre for COVID-19. All patients positive for COVID-19 (by RT-PCR test) were included in the study. Patients positive by COVID antigen reports only were excluded from the study. Additionally, patients with incomplete history or incomplete clinical records were excluded too. The study was approved by the University Ethics Committee (reference no. SU/SMS&R/76-A/2022/63. Written consent and history from the patients was taken at the time of sample collection itself.

METHODOLOGY

RT-PCR for COVID-19

Diagnosis for COVID-19 was done by performing RT-PCR in Bio-safety level-2 cabinet at the molecular laboratory, Microbiology Department, Sharda Hospital.

Extraction of viral RNA from nasopharyngeal and oropharyngeal swabs was done manually using QIAamp Viral RNA Mini Kit, Germany. The amplification of extracted RNA was performed by using COVID-19 RT PCR Kit from Biogenix Inc. Pvt. Ltd., targeting the specific sequence which is conserved and encodes the ORF 1ab gene and the nucleoprotein N gene.

Haematological/Laboratory Parameters

Laboratory parameters including CRP and ferritin were determined by automated methods which included iCHROMA-II automated machine and VITROS 5600 analyzer respectively. Other parameters like D-dimer and IL-6 were evaluated by using semi-automated methods including coagulometer and ELISA respectively.

Grouping of Ct Values

The Ct values were arbitrarily categorized into three groups as:

Group 1: ≤ 20 (low)

Group 2: >20-30 (medium)

Group 3: >30-35 (high)

Statistical Analysis

The data collected was analysed using SPSS-21 software. Categorical variables were presented as numbers and percentage. Two-tailed t-test was used to calculate the p-value for association between Ct value and co-morbidities along with age and gender. P- value of <0.05 was considered insignificant. Karl Pearson’s coefficient was used to find out the correlation between Ct value and laboratory parameters.

RESULTS

A total of 212 patients were enrolled in the study, out of which males outnumbered females. Young patients in the age group between 31 to 60 years were found to be in majority as shown in table 1.

Table 4.1: Demographic Profile of Patients

Demographic character	Frequency (n)	Percentage (%)
Gender		
Males	154	72.6
Females	58	27.4
Age (years)		
<= 30	10	4.7
31- 60	118	55.7
>60	84	39.6

The underlying co-morbidities included chronic diseases like diabetes mellitus, hypertension, cardiac and respiratory illnesses. The distribution is shown in table 4.2

Table 4.2: Chronic Diseases among Patients

Chronic diseases	No. of Patients	Percentage (%)
Diabetes mellitus	36	17
Hypertension	48	22.6
Cardiac disease	26	12.3
Respiratory disease	34	16

The Ct values were arbitrarily categorized into three groups of: low, medium and high and the patients grouped accordingly as shown in table 4.3.

Table 4.3: Distribution of Patients among Various Ct Groups

Groups	Cycle threshold value	No. of patients (n)	Percentage (%)	Mean Ct value ± SD*
1	≤20 (low)	66	31.3	17.51 ± 1.67
2	>20-30 (medium)	126	59.4	25.3 ± 2.66
3	>30-35 (high)	20	9.4	33.3 ± 1.14

*SD= Standard deviation

Association of Ct value with demographic characters and co-morbidities was assessed and no significant association was seen between any one of them except that with cardiac illness where it was found to be significant (table 4).

Table 4.4: Association of Ct Value with Demographic Characters and co-Morbidities

Characteristic	Group 1, n (%)	Group 2, n (%)	Group 3, n (%)	P-value
Males	52 (78.8)	82 (65.1)	20 (100)	
Females	14 (21.1)	44 (34.9)	0 (0)	0.09
Age (mean)	56.33	56.63	56.70	0.99
Diabetics	10 (15.2)	24 (19)	2 (10)	0.54
Hypertensive	16 (24.2)	26 (20.6)	6 (30)	0.61
Cardiac patients	8 (12.1)	12 (9.5)	6 (30)	0.03
Respiratory illness	14 (21.2)	18 (14.3)	2 (10)	0.35

The association between haematological markers and Ct values (table 5) also came out to be non-significant except for IL-6, where it was significant, indicating the direct relation between viral load and cytokine storm.

Table 4.5: Association of Ct Value with Laboratory Parameters

Serological markers	Group 1 (mean±SD)	Group 2 (mean±SD)	Group 3 (mean±SD)	p-value
CRP	79.53 ± 39.23	76.11 ± 43.67	75.86 ± 41.90	0.858
D-dimer	1.11 ± 0.86	1.27 ± 1.17	1.14 ± 0.84	0.551
Ferritin	537.61 ± 381.28	490.87 ± 340.88	423.45 ± 365.55	0.420
IL-6	139.69 ± 152.04	149.73 ± 153.31	47.93 ± 29.50	0.016

DISCUSSION

The present study was carried out to determine the role of Ct value in determining the prognosis of COVID-19 patients by finding out its association with age, gender, co-morbidities and haematological parameters. The study revealed that age and gender of COVID-19 patients had no association with the Ct values and hence, with viral load. This is concordant with the studies done by Penney et al. and Abdulrahman et al. which also show no association of Ct values with age and gender [6, 7]. However, Salvatore et al. showed that Ct values were significantly lower in patients under the age of 18 years [8]. Several factors may contribute to the incongruence observed, including variations in sample sizes, demographic characteristics, and methodologies employed across different studies. Additionally, the dynamic nature of the COVID-19 landscape, with evolving viral strains and fluctuating prevalence rates, may impact the consistency of findings. Furthermore, the diverse array of co-morbidities under investigation and their varying impacts on immune responses might contribute to the heterogeneity in results. We found no association between the Ct value and the comorbidities like diabetes, hypertension and respiratory illness, which is concordant with the study done by AlBahrani et al [9]. Few studies, on the other hand, have shown a significant association between Ct values and co-morbidities like diabetes, hypertension, lung and cardiac disease [5, 10, 11]. This may be because of the difference in timing of sample collection and admission to the hospital. However, we found a significant association of Ct value with cardiac illness. This finding aligns with the established understanding that individuals with underlying cardiovascular conditions may be more susceptible to severe outcomes when infected with the virus. Lower Ct values often indicate a higher viral load, suggesting a potentially more robust and aggressive viral replication within the host. The intricate interplay between the virus and the cardiovascular system is well-documented, with COVID-19 exacerbating pre-existing cardiac issues and, in some cases, triggering myocardial injury. The observed link between lower Ct values and cardiac illness in our study underscores the importance of early identification and targeted intervention in individuals with a heightened viral burden, particularly those with underlying cardiovascular comorbidities various researches have also determined the association between Ct value and haematological indicators like CRP, ferritin and D-dimer in patients with COVID-19 infection. The results of these studies show that as the Ct values decrease, the values of haematological indicators increase and hence there is a significant co-relation between the two [12, 13,14]. This is however not in much agreement with our study results. On the other hand, our results are supported by studies like that done in Bengaluru where they also found no association between the Ct value and increased levels of D-dimer [15]. However, there was a significant association between IL-6 and Ct value as per our study indicating a direct relation between viral load and cytokine storm.

Our study has few limitations. First, we did not follow up patients for the repeat Ct values and considered only one time value to find correlation between various parameters. Also, the Ct values were based on tests at the time of admission, but the patients might be admitted at days different from the symptom onset and thus both Ct values and haematological values might be influenced by the time of getting admitted with respect to symptom onset.

As we reflect on the implications of our study, it becomes evident that the role of Ct values in the management of COVID-19 patients is more nuanced than initially postulated. The absence of a straightforward association underscores the need for continued research efforts to unravel the complexities of this viral illness. First and foremost, future studies should aim to elucidate the factors contributing to the variability in Ct values, including the impact of viral mutations, host immune responses, and the timing of sample collection. The intricate interplay of viral dynamics, host factors, and comorbidities necessitates a comprehensive risk assessment that extends beyond a singular laboratory parameter. Identifying more robust predictors of severe outcomes remains a critical avenue for future research.

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Conflict of Interest: Nil

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