IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 20, Issue 4 Ser.3 (April. 2021), PP 26-31
www.iosrjournals.org

Correlation between Cycle Threshold Values, Co-morbid Conditions, and Outcome in RT-PCR-Positive COVID-19 Patients

Dr Jyoti Iravane[1], Dr Akshay Karyakarte[2], Dr Anil Gaikwad [3], Dhaval Khatri[4], Maitrik Dave[5], Ganesh Korhale[6]

[1,2,3,4,5,6]*Department of Microbiology, Government Medical College, Aurangabad, Maharashtra, India*

Abstract:
Background *Emerging and re-emerging pathogens pose global public health challenges, like the ongoing COVID-19 pandemic. Viral load of SARS CoV-2 loosely correlates with the ΔCt value of an RT-PCR assay, and as such could act as a marker for it. The ΔCt value can thus be useful in predicting the outcome of a patient in presence of co-morbid conditions.*
Material and Methods *Cross-sectional study where nasopharyngeal swabs from suspected COVID-19 patients were tested for the presence of SARS CoV-2 RNA using RT-PCR. The ΔCt values of screening and confirmatory assay of RT-PCR positive samples were noted and analyzed with respect to the demographic details and details of pre-existing co-morbid conditions of RT-PCR positive patients, if any.*
Results*: Total 12298 were tested, of which 1705 were RT-PCR positive in a period of nine months, from April 2020 to December 2020. M: F ratio was 1.27: 1, and 74.90% patients did not have any pre-existing co-morbid conditions. The case fatality rate was 24.63%. One-way ANOVA test showed that the variation of ΔCt value in different age groups, and number of co-morbidities to be significant among the two outcome groups. Pearson's correlation showed significant inverse correlation between ΔCt value, and pre-existing co-morbid conditions and; ΔCt value and outcome.*
Conclusion: *ΔCt value can be utilized as a marker for predicting the outcome in COVID-19-positive patients with pre-existing co-morbid conditions. This is particularly useful in resource-constrained settings for early intervention and mitigation of poor outcome in such cases.*
Key Word*: SARS CoV-2, COVID-19, RT-PCR, co-morbid conditions, outcome.*

Date of Submission: 22-03-2021 Date of Acceptance: 06-04-2021

I. Introduction

Emerging and re-emerging pathogens have always been global public health challenges [1]. In December, 2019, a series of pneumonia cases of unknown cause emerged in Wuhan, Hubei, China, with clinical presentations greatly resembling viral pneumonia [2]. On 11th February 2020, the World Health Organization announced the official name for the disease as the coronavirus disease 2019 (COVID-19), while the International Committee on Taxonomy of Viruses (ICTV) officially named it as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), owing to the genetic likeliness to the coronavirus responsible for the SARS outbreak of 2003 [3, 4]. Being a novel disease, the course of the clinical course was unknown, initially engendering various theories predicting the outcome. A consistent correlation was however found between high viral load of SARS-CoV-2 and poor prognosis [5]. Also, the presence of chronic co-morbidities is a clinical risk factor for a severe or fatal outcome associated with COVID-19 [6]. The diagnosis of COVID-19, like any viral disease, is primarily made using Reverse-transcription Polymerase Chain Reaction (RT-PCR) [7]. The viral load of SARS-CoV-2 is a marker of disease severity, and can predict the risk of death [8]. Although various factors prohibit the quantitation of viral load, the Cycle threshold (ΔCt) value, which is readily calculable with RT-PCR, can act as a coarse marker of viral load in any given sample [9]. As many laboratories carrying out RT-PCR, especially in remote locations, lack the ability to estimate viral load, the ΔCt values of RT-PCR results can justly be used as a coarse marker for viral load. The present study was undertaken with an objective of studying the correlation between ΔCt value and outcome of a patient, and their correlation, if any, with the presence of co-morbid conditions.

II. Material and Methods

The present study is a Cross-sectional study including patients with Severe Acute Respiratory Illness (SARI) from a Dedicated COVID Hospital (DCH), who were tested for the presence of SARS COV-2 RNA using Reverse Transcriptase Polymerase Chain Reaction (RT-PCR), in a period of nine months, from April 2020

to December 2020. The nasopharyngeal samples collected from such patients were transported, in cold chain and triple-layer packaging, to the Viral Research and Diagnostic Laboratory, Department of Microbiology with minimal delay. Ribonucleic Acid (RNA) Extraction was carried out by both, the manual and automated technique, with extraction control. The extracted RNA was then subjected to real time RT-PCR, with the amplification of extraction control determining the validity of the reaction. Various kits approved for use by the Indian Council of Medical Research (ICMR) were used for RT-PCR according to the then-current government guidelines. The targets for amplification were E-gene or S-gene as a screening assay, and RdRP gene, OFR1b gene, or N-gene as a confirmatory assay, according to the kit used. The Cycle Threshold for positivity was determined as per the kit literatures.

The ΔCt values of samples turning out positive by RT-PCR in screening as well as confirmatory assays were noted. Confirmed first-time-positive samples were included in the study whereas those with inconclusive test results were excluded. Additionally, samples sent for repeat testing were also excluded. Patients whose pre-existing co-morbid conditions could not be traced were also excluded. Demographic details of SARS CoV-2 RT-PCR positive patients, along with any pre-existing co-morbid conditions were obtained from the institutional medical records section. The data obtained were compiled using Microsoft® Excel for Mac. Statistical tests of significance like one-way ANOVA and Pearson's correlation were used to analyze the data, in IBM SPSS v26 software.

III. Results

The present study included a total of 12298 patients admitted in various wards of a Dedicated COVID-19 Hospital (DCH). Out of these, 1705 (13.86%) patients turned out positive by RT-PCR. Positivity rate was found higher in male patients (n = 954; 55.95%) as compared to females (n = 751; 44.05%) [Table 1]. Majority of the patients belonged to the 18-59-year age group (n = 1175; 68.91%) [Table 2].

Table 1: Shows sex-wise distribution of patients

Co-morbidities	None	One	Two	Three	>Four	Total
Male	711	156	65	15	7	954
	41.70%	9.15%	3.81%	0.88%	0.41%	55.95%
Female	566	103	62	17	3	751
	33.20%	6.04%	3.64%	1.00%	0.18%	44.05%

Table 2: Shows age-wise distribution of patients

Comorbidities	None	One	Two	Three	>Four	Total
1-17 Years	41	3	0	0	0	44
	2.40%	0.18%	0.00%	0.00%	0.00%	2.58%
18-59 Years	960	141	53	16	5	1175
	56.30%	8.27%	3.11%	0.94%	0.29%	68.91%
> 60 Years	276	115	74	16	5	486
	16.19%	6.74%	4.34%	0.94%	0.29%	28.50%

Pre-existing co-morbid conditions were numerically noted for each patient [Table 3, Chart 1]. 74.90% of the patients did not have any pre-existing co-morbid conditions (n = 1127). 15.19% had one co-morbid condition (n = 259), whereas, 25.10% of the patients had multiple co-morbid conditions. The ΔCt value of the positive samples for screening and confirmatory assay were noted; if booth values differed, the value of confirmatory assay was used. 53.85% patients succumbed when the ΔCt value was less than 10, whereas 81.63% recovered when it was over 30. [Table 4, Chart 2]. A ΔCt value of <25 was taken as low, purportedly corresponding with a high viral load. [10] On comparing the ΔCt values with the pre-existing co-morbid conditions, 64.68% patients had a ΔCt value ≥ 25 in absence of any pre-existing co-morbid conditions, whereas 52.38% had ΔCt value < 25 in the presence of more than three co-morbid conditions [Table 5, Chart 3]. 75.37% of the patients recovered and were subsequently discharged (n = 1285). 24.63% of the patients succumbed (n = 420). Table 3 shows the number of pre-existing co-morbid conditions among COVID-19 Positive patients. Patients succumbing to COVID-19 showed a steady rise in the number of pre-existing co-morbid conditions. Conversely, most of the recovered patients had ≤1 pre-existing co-morbid condition (n = 1213; 71.14%). Pearson's correlation was used to study the statistical significance of this correlation.

Table 3: Shows pre-existing co-morbid conditions among COVID-19-Positive patients

Pre-Existing Co-Morbid Conditions	None	One	Two	Three	Four	Five	TOTAL
Succumbed	189	134	68	21	7	1	420
	14.80%	51.74%	53.54%	65.63%	77.78%	100.00%	24.63%
Recovered	1088	125	59	11	2	0	1285
	85.20%	48.26%	46.46%	34.38%	22.22%	0.00%	75.37%
Total	1277	259	127	32	9	1	1705
	100%	100%	100%	100%	100%	100%	100%

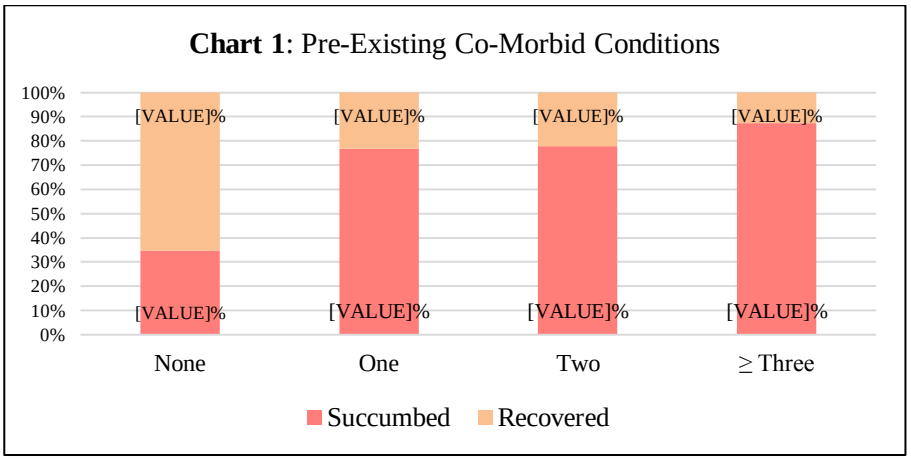


Table 4: Shows ΔCt Value Among COVID-19 Positive Patients

ΔCt Value	≤10	11 to 20	21 to 30	>30	TOTAL
Succumbed	7	104	239	70	420
	53.85%	31.90%	24.26%	18.37%	24.63%
Recovered	6	222	746	311	1285
	46.15%	68.10%	75.74%	81.63%	75.37%
Total	13	326	985	381	1705
	100%	100%	100%	100%	100%

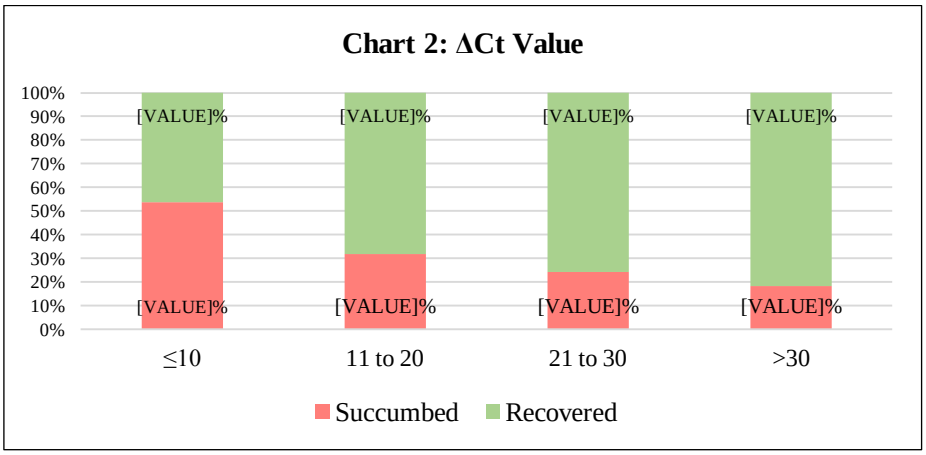
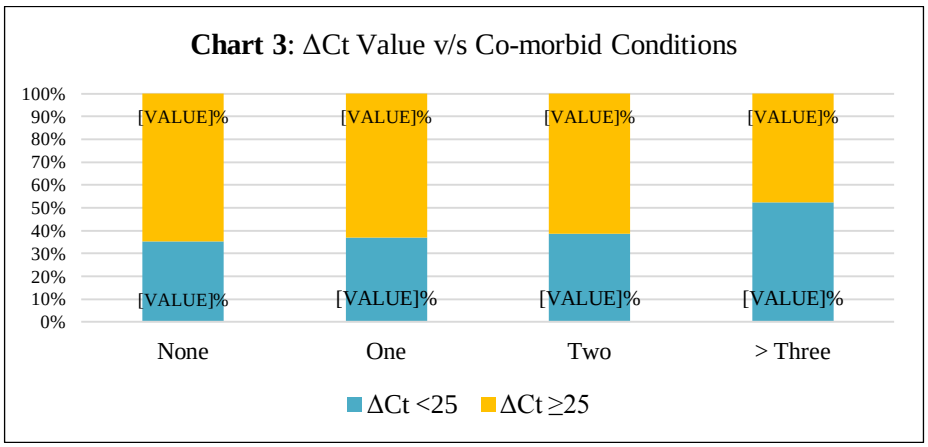


Table 5: Shows ΔCt Value Among COVID-19 Positive With Patients Pre-Existing Co-Morbid Conditions

Pre-Existing Co-Morbid Conditions	None	One	Two	Three	Four	Five	TOTAL
<25	451	96	49	16	5	1	631
	35.32%	37.07	38.58%	50.00%	55.56%	100%	37.01
≥25	826	163	78	16	4	0	1074
	64.68%	62.93	61.42%	50.00%	44.44%	0%	62.99
Total	1277	259	127	32	9	1	1705
	100%	100%	100%	100%	100%	100%	100%



One-way ANOVA test was applied to study the variation among ΔCt values, different age groups, and number of co-morbidities between the two outcomes that is, recovery and death. Descriptive Statistics [Table 6] for mean, standard deviation, standard error of mean and 95% confidence interval were calculated to spot the outliers or missing values before applying one-way ANOVA. Levene's test was applied to test the homogeneity of the data. There was statistically significant difference ($p < 0.05$) between groups, and within groups, as determined by one-way ANOVA test (Table 7 – ΔCt value: $F = 21.471$, $p = 0.000$; Age: $F = 254.18$, $p = 0.000$; Number of co-morbid conditions: $F = 270.748$, $p = 0.000$).

Table 6: Showing descriptive analysis before applying ANOVA

		N	Mean	Standard Deviation	Standard Error	95% Confidence Interval for Mean		Min	Max
						Lower Bound	Upper Bound		
ΔCt Value	Recovered	1285	26.158	5.727	0.160	25.845	26.471	8	40
	Succumbed	420	24.655	5.907	0.288	24.088	25.221	8	39
	Total	1705	25.788	5.806	0.140	25.512	26.064	8	40
Age	Recovered	1262	42.792	17.118	0.482	41.847	43.738	3	94
	Succumbed	418	57.816	15.358	0.751	56.339	59.292	3	100
	Total	1680	46.530	17.913	0.437	45.673	47.388	3	100
Co-morbidities	Recovered	1285	0.221	0.578	0.016	0.189	0.253	0	4
	Succumbed	420	0.871	0.993	0.0484	0.776	0.967	0	5
	Total	1705	0.381	0.757	0.018	0.345	0.417	0	5

Table 7: Shows one-way Analysis of Variance (ANOVA)

		Sum of Squares	dF	Mean Square	F	Significance
ΔCt value	Between Groups	715.27	1	715.27	21.471	0.000
	Within Groups	56731.871	1703	33.313		
	Total	57447.141	1704			
Age	Between Groups	70870.029	1	70870.029	254.18	0.000
	Within Groups	467856.423	1678	278.818		
	Total	538726.452	1679			
Co-morbidities	Between Groups	133.91	1	133.91	270.748	0.000
	Within Groups	842.29	1703	0.495		
	Total	976.199	1704			

Pearson's correlation co-efficient was used to study whether a linear correlation existed between ΔCt Value and number of pre-existing co-morbid conditions, and outcome [Table 6 and 7]. A negative correlation existed between ΔCt Value and number of pre-existing of co-morbid conditions (Pearson correlation = -0.068, $p = 0.005$), as well as outcome (Pearson correlation = -0.112, $p = 0.000$). The negative Pearson value implies that a low ΔCt Value corresponds to higher number of pre-existing co-morbid conditions, and also an increasing correlation with a poor outcome, i.e., death.

A Pearson's correlation co-efficient was also used to study whether a linear correlation existed between Number of co-morbid conditions and outcome (Table 8). A positive correlation existed between number of co-morbid conditions and outcome (Pearson correlation=0.370, p=0.000). The positive Pearson value implies that as the number of pre-existing co-morbid conditions increase the probability of death due to COVID-19 increases.

Table 8: Correlation Between ΔCt Value and Number of Pre-Existing Co-Morbid Conditions

		ΔCt Value	Co-morbidities
ΔCt Value	Pearson Correlation	1	-0.068*
	Significance (2-tailed)		0.005
	N	1705	1705
Co-morbidities	Pearson Correlation	-0.068*	1
	Significance (2-tailed)	0.005	
	N	1705	1705
* Correlation is significant at the 0.01 level (2-tailed).			

Table 9: Correlation Between ΔCt Value and Outcome

		ΔCt Value	Outcome
ΔCt Value	Pearson Correlation	1	-0.112*
	Significance (2-tailed)		0.000
	N	1705	1705
Outcome	Pearson Correlation	-0.112*	1
	Significance (2-tailed)	0.000	
	N	1705	1705
* Correlation is significant at the 0.01 level (2-tailed).			

Table 10: Correlation Between Number of Co-Morbid Conditions and Outcome

		ΔCt Value	Outcome
Outcome	Pearson Correlation	1	0.370*
	Significance (2-tailed)		0.000
	N	1705	1705
Co-morbidities	Pearson Correlation	0.370*	1
	Significance (2-tailed)	0.000	
	N	1705	1705
* Correlation is significant at the 0.01 level (2-tailed).			

IV. Discussion

The present DCH admitted and tested a total of 12298 patients during the nine months of the study period. Of those 1705 patients turned out positive by RT-PCR, with a percent positivity = 13.86%. Of these 1705 RT-PCR positive cases, 954 were male (55.95%) and 751 were female (44.05%). The Male-to-Female ratio was 1.27: 1. Additionally, majority of the patients belonged to the 18-59-year age group (n = 1175; 68.91%). Similar findings were obtained in an initial study in Wuhan, Hubei, China by Xiao *etal* where 63.5% patients were below the age of 65 years, and the positivity showed sight male preponderance [11]. These findings were also similar to the study in Jaipur, Rajasthan, India by Bhandari *etal* in which 80.9% of the patients were younger than 60 years of age, and showed male preponderance [12]. Since the demographic details have no impact on the parameters of the present study, the findings were simply tabulated, without further statistical analysis. Pre-existing co-morbid conditions, if any, in all the RT-PCR Positive patients were obtained, and the number of co-morbid conditions in each of them was noted. Most of the patients did not have any pre-existing co-morbid conditions (n=1127; 74.90%). The mortality rate of COVID-19 in the present DCH was 3.42% (n = 420 of 12298). Similar findings were obtained in a study in China by Baud *etal* where mortality rate was 3.6% [13]. The global mortality rate is found to be 4.7%, while that in Italy was 10.8% [14]. The present study substantiated the findings of Jain *etal*, who reported the mortality in India to be lower than that in the western world [15]. The CFR turned out to be 24.63% (n = 420 of 1705), this also correlated favorably with other national and international studies. Consequently, the rate of recovery in the present DCH was 75.37% (n = 1285 of 1705).

The variation among ΔCt values, different age groups, and number of co-morbidities between the two outcomes, recovery, and death, was determined using One-way ANOVA test. The test showed significant within and between group variation ($p<0.05$). On obtaining a significant ANOVA test, the Pearson's Correlation Co-efficient test was then applied in order to study the type of correlation between ΔCt values and number of co-morbidities and their correlation with the two outcomes, recovery, and death. In both these cases, the Pearson's Correlation Coefficient was negative, indicating an inverse correlation, which was found to be significant. This was followed by the positive linear correlation between number of pre-existing co-morbid conditions in a COVID—19 positive patient and the outcome. These findings indicate that a COVID-19 positive patient is more likely to succumb as the ΔCt value decreases, and also as the number of pre-existing co-morbid conditions increase. It can therefore be inferred that a low ΔCt value in a patient with more pre-existing co-morbid conditions is more likely to succumb, while a high ΔCt value in the presence of fewer or no pre-existing co-morbid conditions is likely to survive.

V. Conclusion

The ΔCt value of RT-PCR positive patients can be utilized as a marker for predicting the outcome in COVID-19-positive patients with pre-existing co-morbid conditions. This is particularly useful in resource-constrained settings, where facilities and consumables for determination of viral load are scant. As these findings are available to the clinician along with the positive RT-PCR report itself. An early intervention can be instituted in such cases to mitigate a poor outcome.

References

[1]. Gao GF. From "A" IV to "Z" IKV: attacks from emerging and re-emerging pathogens. Cell. 2018 Mar 8;172(6):1157-9.

[2]. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, Zhao X, Huang B, Shi W, Lu R, Niu P. A novel coronavirus from patients with pneumonia in China, 2019. New England Journal of Medicine. 2020 Jan 24.

[3]. Coronavirus Disease 2019 (COVID-19), Centers for Disease Control and Prevention, 01 September 2020. [online] available from https://www.cdc.gov/coronavirus/2019-ncov/cdcresponse/about-COVID-19.html#:~:text=On%20February%2011%2C%202020%2C%20the,and%20'D'%20for%20disdise.

[4]. Naming the coronavirus disease (COVID-19) and the virus that causes it; World Health Organization, 11 February 2020. [online] available from https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/naming-the-coronavirus-disease-(covid-2019)-and-the-virus-that-causes-it#:~:text=ICTV%20announced%20%E2%80%9Csevere%20acute%20respiratory,the%20SARS%20outbreak%20of%202003.

[5]. Liu Y, Yan LM, Wan L, Xiang TX, Le A, Liu JM, Peiris M, Poon LL, Zhang W. Viral dynamics in mild and severe cases of COVID-19. The Lancet Infectious Diseases. 2020 Mar 19.

[6]. Zhou Y, Yang Q, Chi J, Dong B, Lv W, Shen L, Wang Y. Comorbidities and the risk of severe or fatal outcomes associated with coronavirus disease 2019: A systematic review and meta-analysis. International Journal of Infectious Diseases. 2020 Jul 25.

[7]. Bullard J, Dust K, Funk D, Strong JE, Alexander D, Garnett L, Boodman C, Bello A, Hedley A, Schiffman Z, Doan K. Predicting infectious SARS-CoV-2 from diagnostic samples. Clinical Infectious Diseases. 2020 May 22.

[8]. Fajnzylber J, Regan J, Coxen K, Corry H, Wong C, Rosenthal A, Worrall D, Giguel F, Piechocka-Trocha A, Atyeo C, Fischinger S. SARS-CoV-2 viral load is associated with increased disease severity and mortality. Nature communications. 2020 Oct 30;11(1):1-9.

[9]. Rhoads D, Peaper DR, She RC, Nolte FS, Wojewoda CM, Anderson NW, Pritt BS. College of American Pathologists (CAP) microbiology committee perspective: caution must be used in interpreting the cycle threshold (Ct) value. Clinical Infectious Diseases. 2020 Aug 12.

[10]. Magleby R, Westblade LF, Trzebucki A, Simon MS, Rajan M, Park J, Goyal P, Safford MM, Satlin MJ. Impact of SARS-CoV-2 viral load on risk of intubation and mortality among hospitalized patients with coronavirus disease 2019. Clinical infectious diseases. 2020 Jun 30.

[11]. Xiao AT, Tong YX, Gao C, Zhu L, Zhang YJ, Zhang S. Dynamic profile of RT-PCR findings from 301 COVID-19 patients in Wuhan, China: a descriptive study. Journal of Clinical Virology. 2020 Jun 1;127: 104346.

[12]. Bhandari S, Bhargava A, Sharma S, Keshwani P, Sharma R, Banerjee S. Clinical profile of covid-19 infected patients admitted in a tertiary care hospital in north India. J Assoc Phys India. 2020 May 1;68: 13-7.

[13]. Baud D, Qi X, Nielsen-Saines K, Musso D, Pomar L, Favre G. Real estimates of mortality following COVID-19 infection. The Lancet infectious diseases. 2020 Jul 1;20(7):773.

[14]. Omer SB, Malani P, Del Rio C. The COVID-19 pandemic in the US: a clinical update. JAMA. 2020 May 12;323(18):1767-8.

[15]. Jain VK, Iyengar K, Vaish A, Vaishya R. Differential mortality in COVID-19 patients from India and western countries. Diabetes & Metabolic Syndrome: Clinical Research & Reviews. 2020 Sep 1;14(5):1037-41.

Dr Jyoti Iravane, et. al. "Correlation between Cycle Threshold Values, Co-morbid Conditions, and Outcome in RT-PCR-Positive COVID-19 Patients."*IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)*, 20(04), 2021, pp. 26-31.