

# Cycle Threshold (CT) Values in COVID-19 Patients Admitted in Intensive Care Unit and Their Outcome: An Observational Study from South Punjab

ZOHAIB ASHRAF<sup>1</sup>, AMINA ASIF<sup>2</sup>, SYED KHIZAR ABBAS RIZVI<sup>3</sup>, UZMA SHAHEEN<sup>4</sup>, MUHAMMAD ILYAS<sup>5</sup>, MUHAMMAD DAUD RAZA<sup>6</sup>, BABAR ABDUL GHAFOR<sup>7</sup>

<sup>1,4</sup>Assistant Professor of Pathology, DG Khan Medical College, Dera Ghazi Khan, Pakistan

<sup>2</sup>Assistant Professor of Pathology, Ameer-ud-Din Medical College/ Post Graduate Medical Institute, / Lahore General Hospital, Lahore, Pakistan

<sup>3</sup>Associate Professor Pathology, Sahiwal Medical College, Sahiwal Pakistan

<sup>5</sup>Assistant Professor of ENT Ameer-ud-Din Medical College/Lahore General Hospital/Post Graduate Medical Institute, Lahore

<sup>6</sup>MBBS, Shahida Islam Medical and Dental College, Lodhran

<sup>7</sup>MSc Statistics, Government College University, Lahore

Corresponding author: Amina Asif, Email: [aminasif79@gmail.com](mailto:aminasif79@gmail.com)

## ABSTRACT

**Introduction:** The World Health Organization declared the COVID-19 epidemic an international public health emergency on January 30th, 2020. Many patients required ICU admission for advanced respiratory support, including high flow nasal oxygen, and non-invasive, and invasive mechanical ventilation. Lower Ct values may be associated with disease severity

**Objective:** To observe the Cycle threshold (Ct) value and outcome of the COVID-19 patients admitted in Intensive Care Unit (ICU).

**Methods:** Nasopharyngeal/ oropharyngeal samples were collected via swabs from suspected COVID-19-positive patients admitted to Corona ICU of Teaching Hospital, Dera Ghazi Khan (DG Khan). Samples were proceeded for RNA extraction and amplification and were declared positive on basis of the S-shaped amplification curve at FAM (ORF1) and ROX channel, and a Ct value of  $\leq 40$ .

**Results:** Out of 59 ICU admissions, 17 were females and 42 males. The highest percentage of mortality was observed in the 60 and above years of age group (78.98%). Ct values were lower in patients who expired as compared to the patients who were discharged (mean 21.052 vs 23.739).

## Conclusion:

Males were more frequently admitted to ICU for management of severe infection by COVID-19 than females. Mortality is associated with increasing age. Cycle threshold (Ct) values were lower in patients who expired than those who recovered

**Keywords:** Cycle threshold, RT-PCR, COVID-19, ICU

## INTRODUCTION

Many patients were identified in Wuhan, China in December 2019 with pneumonia of unknown cause [1], which subsequently lead to the identification of severe acute respiratory syndrome coronavirus (SARS-CoV-2) [2]. The World Health Organization declared COVID-19 an international public health emergency on January 30th, 2020 [3].

Clinical presentation of SARS-CoV-2 infection ranges from asymptomatic, mild to moderate COVID-19, and COVID-19 pneumonia, with some patients requiring intensive care support and, in some cases, leading to death, especially in the elder population. [4,5].

ICU admission for advanced respiratory support, including high flow nasal oxygen, and non-invasive, and invasive mechanical ventilation has remained one of the major challenges faced during the pandemic of COVID-19 [6]. The mortality rate of Covid -19 patients during intensive care has been observed to be above 40% which is notably higher than the 22.0% seen in other patients admitted to ICU with viral pneumonia [7,10].

According to WHO guidelines, the Reverse Transcriptase Quantitative Polymerase Chain Reaction (RT-qPCR) test, which detects SARS-CoV-2 RNA in respiratory samples, is a reliable method for diagnosis of COVID-19 in patients [3]. The RT-qPCR results are either positive or negative [5]. The viral load on the samples is measured by RT-PCR and presented as a Cycle Threshold (Ct) value. A Low Ct value indicates a high viral load and is a strong indication of transmissibility [8]. Ct value (calculated viral load) has been demonstrated to provide beneficial insight into illness status interpretation as a clinical decision support tool [6,7]. Lower Ct values may be associated with disease severity, a longer and worse course of sickness, and worse outcomes, which might provide significant information in predicting a patient's clinical course and prognosis [8]. Active virus replication has been detected and assessed in tissue samples, and it is commonly used to monitor viral respiratory tract infections, clinical course, response to therapy, recovery, and recurrence [9].

The genome of coronaviruses includes a variable number of open reading frames (ORFs) and the size of the genome ranges

between approximately 26,000 and 32,000 bases [11]. The first ORF representing approximately 67% of the entire genome encodes 16 non-structural proteins (nsps), while the remaining ORFs encode accessory proteins and structural proteins. The four major structural proteins are the spike surface glycoprotein (S), a small envelope protein (E), matrix protein (M), and nucleocapsid protein (N) [12,13].

This study was carried out to observe the viral dynamics variation in different age groups and gender with severe COVID disease admitted to the Intensive Care Unit by using Ct value and observing their outcome. Currently, in Pakistan, not much data has been shared to report Ct value distribution in correlation with age and gender among ICU patients.

## METHODOLOGY

This cross-sectional, observational study was conducted on the COVID-19 patients admitted to the Intensive Care Unit of Teaching Hospital, Dera Ghazi Khan from May 2021 to August 2021

Nasopharyngeal or oropharyngeal samples were collected via swabs from suspected COVID-19 patients admitted to Corona ICU of Teaching Hospital, DG Khan. VTM (viral transport medium) vials were labelled with patient names. Samples were collected after wearing full PPEs. After collection, labelled samples and the request form were placed in a plastic bag and put into a container with ice bags. The request form included details of the patient's name, age, gender, national identity card number, mobile number, date of admission to ICU, and COVID-19 number. Samples were transported to the Biosafety Level (BSL) III laboratory of Teaching Hospital DG Khan.

Amplification of samples was carried out by Quant Studio Real-Time PCR. 30  $\mu$ l of PCR mix and 20  $\mu$ l of samples were poured into PCR reaction tubes. PCR reaction tubes were placed into specimen wells of Quant studio RT -PCR machine. Negative and positive controls were set up. FAM(ORF1) and ROX were selected as target genes to be amplified and Cy5 as an internal control. Amplification curves of the specimens were analyzed. Samples were declared positive on basis of the S-shaped

amplification curve at FAM (ORF1) and ROX channel, and a Ct value of  $\leq 40$ .

Data were expressed as mean  $\pm$  SD, median [interquartile range (IQR)], 95% Confidence Interval (CI), or percentages, as appropriate. One sample Wilcoxon signed rank test and Pearson Chi-Square test was applied where applicable. The level of significance was set at  $P < 0.05$

### RESULTS

59 patients were admitted to the COVID ICU of Teaching hospital DG Khan from May 2021 to August 2021 that tested positive for SARS CoV 2 on RT-PCR. Out of these, 17(29%) were females and 42 (71%) were males. The demographic characteristics of the patients are shown in figure 1.

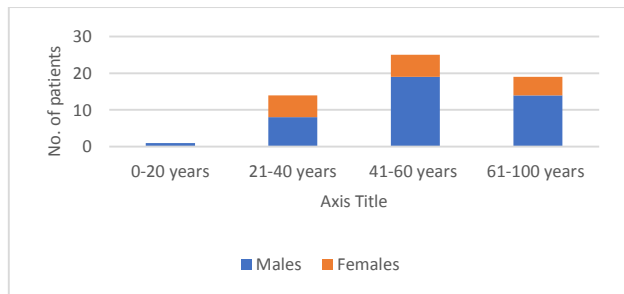


Figure 1: Demographic characteristics of the COVID-19 patients admitted to ICU

37 (62.71%) out of 59 patients expired in the ICU. The outcome of the patients, regarding their gender and age group, admitted to the COVID ICU is given in table 1.

Table 1: Outcome of COVID-19 patients admitted in ICU

Age Groups	Total Count	Expired (%)	Recovered (%)	P-value
< 20 years	1	1 (100)	0	<0.01
21 to 40 years	14	4 (28.57)	10 (71.43)	
41 to 60 years	25	17 (68.00)	8(32.00)	
>60 years	19	15(78.94)	4(21.06)	
Gender				0.694
Male	42	27(64.28)	15(35.71)	
Female	17	10 (58.82)	7(41.17)	

Viral load quantification was performed by using a standard curve equation derived from the serial dilution of the known concentration of the RNA and the Ct value of ORF1. Non-parametric t-test was performed to see the difference in viral load of expired and discharged patients. In total, among expired and discharged patients there was no statistically significant difference in the viral load or density. Nevertheless, the ct value for expired patients was less than those of patients who were discharged. (mean  $21.052 \pm 3.582$  vs mean  $23.739 \pm 2.449$  respectively).

Table 2: Distribution of Ct value among expired and recovered COVID-19 patients' samples positive for ORF1

Ct- Value	Expired patients	Recovered patients
Count	37	22
Mean	21.052	23.739
Standard deviation	3.582	2.449
Variance	12.828	5.996
Minimum	14.52	19.28
1st Quartile	18.295	21.99
Median	21.23	23.655
3rd Quartile	24.4	26.245
Maximum	27.59	28.33
95% CI for Mean	19.858 to 22.246	22.653 to 24.825
95 % CI for Median	19.083 to 22.187	22.050 to 25.666
95 % CI for Standard Deviation	2.913 to 4.652	1.884 to 3.499
P -value	0.334	0.502

Interval plots were drawn for ct value and age groups of expired and discharged patients as shown in figure 2.

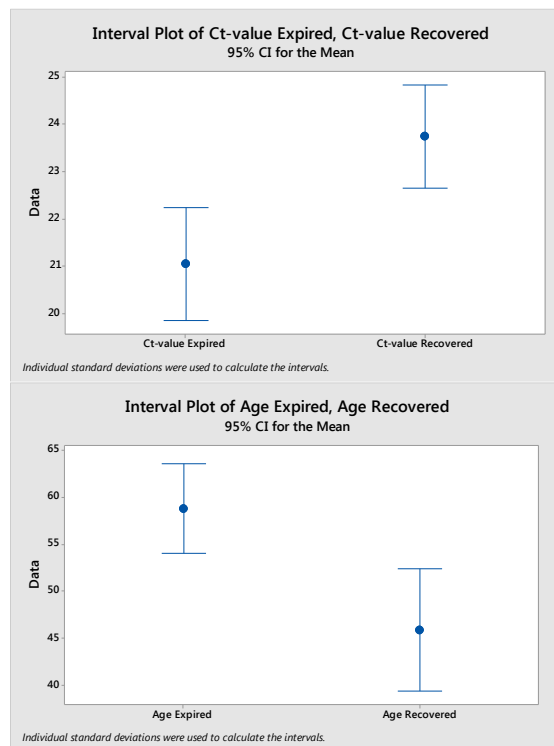


Figure 2: Interval plots of Ct value and age group for expired and discharged COVID-19 patients admitted to ICU

### DISCUSSION

Patients with covid-19 usually presented with fever, cough, and shortness of breath. The most common previous major comorbidities included diabetes, chronic cardiac disease, and chronic non-asthmatic pulmonary disease (6).

In the current study, data from 59 patients were included who were admitted to ICU. Out of these patients, 13 patients were females while 36 were males which shows that males were more frequently admitted to ICU than females. Our study coincides with other studies where males were more frequently admitted to ICU than females (17). In a study conducted in Italy, the rate of admission to ICU was higher for males, compared to females (18).

Among ICU admissions, as shown in Table No.1, the highest percentage of mortality was seen in the 60 and above years of the age group where 15 patients expired out of 19 admissions while maximum recoveries were seen in the 21-40 years of age group. There was only one admission in the below 20 years of the age group who expired. In the far southern district of Dera Ghazi Khan of Punjab, there was a total of 59 ICU admissions during the second wave of the COVID pandemic. Out of these 59 admissions, 37 patients expired, making an ICU mortality percentage of 62.71%. This high mortality percentage is due to multiple reasons, of which notables are initial Hakeem/quack management, late presentation to hospitals, untreated comorbidities, hospital-acquired infections, and untrained ICU staff. In a study conducted in the US, percentages of ICU admissions were lowest among adults aged 20–44 years and highest among those aged 60 & above. Case-fatality percentages increased with increasing age, from no deaths reported among persons aged  $\leq 19$  years to the highest percentages among adults aged 60 and above (19). Table no.2 shows the Ct values of the patients who were admitted to ICU. This table shows that Ct values were lower in patients who expired or showed severity in their disease course with the worst outcome than those who recovered and showed

better outcomes despite ICU admission. The mean Ct values among 37 patients who expired were observed to be 21.051 with a minimum Ct value of as low as 15.52. While in 22 recovered patients mean Ct-value was 23.739 with a minimum Ct value of 19.28. In a study conducted in England, Salvatore et al found that Ct values were significantly lower among those reporting one or more respiratory symptoms compared to those with no respiratory symptoms, and median Ct values were lower among patients with several symptoms, compared to participants who had mild or no symptoms (20). Similarly, in 308 hospitalized adult patients in China, average Ct values were lower in patients who expired than those who recovered (21).

## CONCLUSION

The results of this study conclude that males were more frequently admitted to ICU for management of severe infection by COVID-19 than females. Mortality is associated with increasing age. Cycle threshold (Ct) values were lower in patients who expired than those who recovered. However, this study has a few limitations in that it was conducted on a small scale where only one patient was admitted to ICU below 20 years of age who expired so we cannot comment on the death percentage of this age group. Further studies should be conducted for age group involvement, Ct values, and patient outcome.

**Conflict of Interest:** All authors declare no conflict of interest.

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## REFERENCES

- Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., Zhang, L., Fan, G., Xu, J., Gu, X. and Cheng, Z., 2020. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *The Lancet*, 395(10223), pp.497-506.
- Zhu, N., Zhang, D., Wang, W., Li, X., Yang, B., Song, J., Zhao, X., Huang, B., Shi, W., Lu, R. and Niu, P., 2020. A novel coronavirus from patients with pneumonia in China, 2019. *New England Journal of Medicine*.
- Rakesh Kumar Jha, Pradip Jain, Ranjit S. Ambad, Nandkishor (2020) THE ORIGIN, TRANSMISSION AND MORTALITY OF CORONA VIRUS. *JCR*, 7 (10), 93-96. doi:10.31838/jcr.07.10.23
- Siddiqui, A.A., 2020. The Epidemiology of COVID-19 Novel Corona Virus in Incidence and the Distribution of the Disease across the World.
- Struyf, T., Deeks, J.J., Dinnes, J., Takwoingi, Y., Davenport, C., Leeflang, M.M., Spijker, R., Hooft, L., Emperador, D., Dittich, S. and Domen, J., 2020. Signs and symptoms to determine if a patient presenting in primary care or hospital outpatient settings has COVID-19 disease. *Cochrane Database of Systematic Reviews*, (7)
- Docherty, A.B., Harrison, E.M., Green, C.A., Hardwick, H.E., Pius, R., Norman, L., Holden, K.A., Read, J.M., Donnelinger, F., Carson, G. and Merson, L., 2020. Features of 20 133 UK patients in hospital with covid-19 using the ISARIC WHO Clinical Characterisation Protocol: prospective observational cohort study. *bmj*, 369.
- Guo, Y.R., Cao, Q.D., Hong, Z.S., Tan, Y.Y., Chen, S.D., Jin, H.J., Tan, K.S., Wang, D.Y. and Yan, Y., 2020. The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak—an update on the status. *Military Medical Research*, 7(1), pp.1-10.
- Awadasseid, A., Wu, Y., Tanaka, Y. and Zhang, W., 2020. Initial success in the identification and management of the coronavirus disease 2019 (COVID-19) indicates human-to-human transmission in Wuhan, China. *International Journal of Biological Sciences*, 16(11), p.1846.
- van de Veerdonk, F.L., Netea, M.G., van Deuren, M., van der Meer, J.W., de Mast, Q., Brüggemann, R.J. and van der Hoeven, H., 2020. Kallikrein-kinin blockade in patients with COVID-19 to prevent acute respiratory distress syndrome. *Elife*, 9, p.e57555.
- Armstrong, R.A., Kane, A.D. and Cook, T.M., 2020. Outcomes from intensive care in patients with COVID-19: a systematic review and meta-analysis of observational studies. *Anaesthesia*, 75(10), pp.1340-1349.
- Song, Z., Xu, Y., Bao, L., Zhang, L., Yu, P., Qu, Y., Zhu, H., Zhao, W., Han, Y. and Qin, C., 2019. From SARS to MERS, thrusting coronaviruses into the spotlight. *Viruses*, 11(1), p.59.
- Cui, J., Li, F. and Shi, Z.L., 2019. Origin and evolution of pathogenic coronaviruses. *Nature Reviews Microbiology*, 17(3), pp.181-192
- Zhu, Z., Zhang, Z., Chen, W., Cai, Z., Ge, X., Zhu, H., Jiang, T., Tan, W. and Peng, Y., 2018. Predicting the receptor-binding domain usage of the coronavirus based on kmer frequency on spike protein. *Infection, Genetics and Evolution*, 61, p.183.
- Wiersinga, W.J., Rhodes, A., Cheng, A.C., Peacock, S.J. and Prescott, H.C., 2020. Pathophysiology, transmission, diagnosis, and treatment of coronavirus disease 2019 (COVID-19): a review. *Jama*, 324(8), pp.782-793
- Revi, D., 2020. Pathogenesis, immune response and laboratory diagnosis of severe acute respiratory syndrome associated Coronavirus 2. *Open J Biol Sci*, 5(1), pp.041-046.
- Dramé, M., Teguo, M.T., Proye, E., Hequet, F., Hentzien, M., Kanagaratnam, L. and Godaert, L., 2020. Should RT-PCR be considered a gold standard in the diagnosis of Covid-19? *Journal of Medical Virology*.
- Chen, N., Zhou, M., Dong, X., Qu, J., Gong, F., Han, Y., Qiu, Y., Wang, J., Liu, Y., Wei, Y. and Yu, T., 2020. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *The Lancet*, 395(10223), pp.507-513.
- Iaccarino, G., Grassi, G., Borghi, C., Carugo, S., Fallo, F., Ferri, C., Giannattasio, C., Grassi, D., Letizia, C., Mancusi, C. and Minuz, P., 2020. Gender differences in predictors of intensive care units admission among COVID-19 patients: The results of the SARS-RAS study of the Italian Society of Hypertension. *PloS one*, 15(10), p.e0237297.
- COVID, T.C. and Team, R., 2020. Severe Outcomes Among Patients with Coronavirus Disease 2019 (COVID-19)—United States, February 12–March 16, 2020. *MMWR Morb Mortal Wkly Rep*, 69(12), pp.343-346.
- Salvatore, P.P., Dawson, P., Wadhwa, A., Rabold, E.M., Buono, S., Dietrich, E.A., Reses, H.E., Vuong, J., Pawloski, L., Dasu, T. and Bhattacharyya, S., 2020. Epidemiological Correlates of PCR Cycle Threshold Values in the Detection of SARS-CoV-2. *Clinical Infectious Diseases*.
- Rao, S.N., Manissero, D., Steele, V.R. and Pareja, J., 2020. A narrative systematic review of the clinical utility of cycle threshold values in the context of COVID-19. *Infectious diseases and therapy*, pp.1-14.