Original Article

A closer look at the link between cycle threshold, clinical features and biomarkers: An observational study in COVID-19 patients

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Abstract

Background: Symptoms for severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) appear 2–3 days after exposure to the virus. Being a virus, detection is primarily by polymerase chain reaction as this offers superior sensitivity and specificity. There was a misconception that patients with low cycle threshold (Ct) have severe coronavirus disease (COVID), and for individuals with higher Ct, it is the other way around. The prognosis for COVID was derived from various biomarkers and physicians heavily relied on them. **Materials and Methods:** A cross-sectional study spanning a duration of 2 years was conducted at a tertiary care centre in western India. A total of 201 individuals were included and the correlation between Ct, clinical features and biomarkers was studied. **Results:** In the E-gene, 43.28% had lower Ct values and 40.79% had low Ct values in the RdRp gene. 50% of all patients had diabetes, with 60% being between the ages of 61 and 80. 54.1% of hypertension patients belonged to ages between 61 and 80. 90.54% of COVID-positive individuals had lactose dehydrogenase levels ranging from 440 to 760. 79% of patients had a procalcitonin value of more than one but less than six. 79.1% of patients had an erythrocyte sedimentation rate between 36 and 90. **Conclusion:** Ct value though has a research value; it is a poor prognostic marker when compared to the various biomarkers that have been studied earlier. We cannot conclusively state that all our findings are accurate due to a lack of data but further research into the prognostic value of Ct should be conducted which will help in the ongoing scenario.

Keywords: Biomarkers, clinical features, COVID-19, cycle threshold

Introduction

Since ancient times respiratory diseases have been a part of the society, with a multitude of etiologies associated with respiratory illnesses.[1] Many viruses enter the respiratory tract via aerosols, droplets or droplet nuclei to induce a localised illness, but some also cause systemic illness.[2]

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) has captured the interest of the entire globe. The symptoms appeared 2–3 days after the patient was exposed to CoV. Hospitals in India were turned into coronavirus disease-designated (COVID) centres with isolation facilities during the first wave of the COVID-19 epidemic.[3]

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In terms of direct identification of causal organism genetic material, polymerase chain reaction (PCR) methods offer superior sensitivity and specificity. CoV contains RNA-type nucleic material, hence diagnosis needed reverse transcriptase-PCR (RT-PCR).[4-6] Reporting of RT-PCR includes various genes and cycle threshold (Ct) values, estimating the viral load. Low or high Ct indicated high or low viral load. There was a misperception that patients having lower Ct needed intensive care unit (ICU) management while those with higher Ct could be treated on an out-patient basis.[7]

Disease prevalence has decreased as the illness has advanced, but the aftereffects are still felt by the masses. When a patient contracts a COVID infection, the symptomology is difficult to forecast using existing diagnostic techniques.

Comorbidities, such as hypertension (HTN), cardiovascular illness, and type 2 diabetes mellitus, as well as immunocompromised individuals, had greater severity of COVID-19 infection than non-comorbid patients; however, there was little evidence to support the notion.[8] Nonetheless, there is a significant risk of ICU hospitalisation and death in all comorbid patients, not only those with COVID-19 infection.[9] Therapy management during hospitalisation is also critical in patient care and recovery of all diseases.

The severity of the infection and the progress of the patient are determined by the treating physician based on the immunological markers that become aberrant or increased during the illness. The severity of the infection was estimated based on those marker test results at the time of admission, and treatment effect was demonstrated by changes in that marker becoming within normal range.

This study was formulated to investigate the connotations concerning Ct values from RT-PCR for COVID-19 and clinical symptoms in a COVID patient, as well as to see whether there are any additional links between Ct and biomarkers found in COVID.

Materials and Methods

A cross-sectional study was conducted in a tertiary care centre in western India from November 2020 to October 2022. The study included 201 individuals with COVID-positive RT-PCR between the ages of 15 and 65 years, while others were omitted.

The patient's detailed history, clinical characteristics, and concomitant disorders have all been documented. Nasopharyngeal swab was collected from suspected patients in a viral transport medium and processed for RT-PCR. SARS-CoV2 was detected using a kit validated and approved by Indian Council for Medical Research (ICMR); looked for the presence of the Envelop (E)-gene and RNA-dependent RNA polymerase (RdRp) genes. Ct values (range 0–38) were acquired from the COVID-positive individuals; any Ct value more than 38 was considered negative. The immunological, haematological and biochemical markers, which include fibrinogen, immunoglobulin M (Ig-M), interleukin-6, C-reactive protein (CRP), Ferritin, D-dimer, procalcitonin, Ig-G, lactose dehydrogenase (LDH), and complete blood count, are assessed for changes in values.

SpO2, respiratory rate, the presence of dyspnoea, mental state, and the need for ventilator assistance will be used to determine the severity of the clinical situation. Any parameter indicating organ failure will be recorded as well.

Statistical analysis

Data were entered in an Excel sheet and analysed using SPSS v23 (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.).[10] Tests of statistical significance such as Chi-square and paired *t*-test were used.

Ethical approval

Study approved by institutional ethics sub-committee under reference number I.E.S.C/268/2021 dated 23rd December 2021.

Patient consent

Written informed consent was taken from the patient regarding data collection and research purposes.

Results

During the research period, 201 COVID patients' data were obtained. Table 1 depicts the gender and age distribution. The overall scattering configuration for either gender was similar, but the tally for the commonest age group diverged. The majority of males were between the ages of 31 and 40 years, while the majority of ladies were between the ages of 71 and 80 years.

RT-PCR was used to estimate the duration of SARS-CoV2 clearance in respiratory specimens in these individuals who were assessed for COVID. In addition, patients' COVID status was classified into three categories owing to the Ct value of patients: low (Ct ≥ 30), intermediate (25 < Ct < 30) and high (Ct ≤ 25). Patients having low Ct are likely to develop COVID and vice versa. Figure 1 depicts how these individuals were classified based on their Ct value. The genes used to establish the Ct value for classification are E-gene and RdRp gene.

Table 1: Demographic distribution of the patients

Gender vs age group distribution *n* (%)			
Age group	**Male (*n*=123)**	**Female (*n*=78)**	**Total (*n*=201)**
21–30 Years	1 (0.81)	0	1 (4.97)
31–40 Years	44 (35.77)	20 (25.64)	64 (31.84)
41–50 Years	18 (14.63)	15 (19.23)	33 (16.41)
51–60 Years	6 (4.87)	8 (10.25)	14 (6.96)
61–70 Years	11 (8.94)	0	11 (5.47)
71–80 Years	32 (26.01)	33 (4.23)	65 (32.33)
81–85 Years	11 (8.94)	2 (2.56)	13 (6.46)

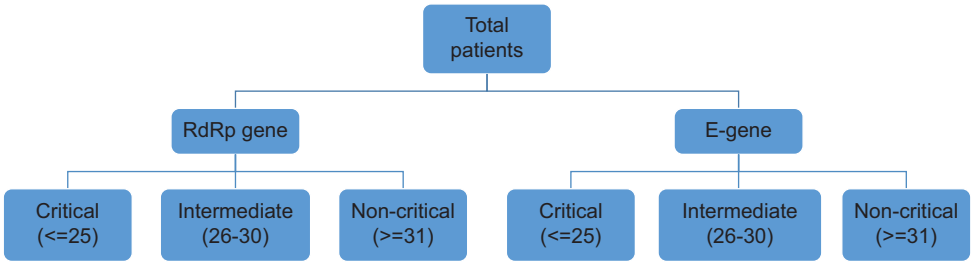

Figure 1: Distribution of patients based on E-gene and RdRp gene

Of the 201 patients, 43.28% (87) had lower Ct values in E-gene and 40.79% (82) had low Ct values in the RdRp gene. According to the study, 40% of the patients had a lower Ct value, indicating a serious COVID status. In both genes, about 32.8–30.8% of patients (62–66) had intermediate Ct values, while 23.8–28.3% of patients (48–57) had high Ct values [Figure 1].

Table 2 depicts the age distribution of patients in relation to their comorbid condition, admission status, SpO2 level and oxygenation demand. It was discovered that 50% of all patients had diabetes, with 60% being between the ages of 61 and 80 years. Similarly, 54.1% of HTN patients belonged to ages 61 and 80 years. This shows that the concomitant condition is prevalent in people of this age range. These patients' admission status was divided into two categories, ICU and Ward; 82% of the patients in the study were in the ICU, while the remaining 18% were in the general ward. Again, the 61–80 years age group had the largest number of ICU stays, at 40% (67), although the fledgling age group 21–40 years also had 34.5% (57) patients. Likewise, in invasive oxygenation support, two patient groups (21–40 and 61–80 years) were heavily represented.

The clinical diagnosis in this investigation was based on four symptoms of the patients. The Venn diagram in Figure 2 depicts the distribution of patients based on four identified signs. The most common was breathlessness in 94.5% of instances (190), followed by fever in 48.25% of cases (97). Accounting for just 6–10% of cases were loss of smell and taste. Only in six instances, all four symptoms were present.

Eventually, the most populated range was determined by diagnosing several blood indicators in all COVID-positive individuals. The LDH test suggests tissue injury. LDH is an enzyme with a typical blood level of 140–280 U/L in healthy people. We found that 90.54% of COVID-positive individuals had LDH levels ranging from 440 to 760.

The procalcitonin test determines the severity of bacterial infection. Bacterial infection is defined as a procalcitonin level of more than 0.25 ng/mL. In this case, 79% of patients had a procalcitonin value of more than one but less than six. D-dimer is the breakdown product of fibrin that has been cross-linked (by factor XIII). It indicates that the haemostatic system is still active. The standard concentration of D-dimer is 250 ng/mL, or 0.4/mL. The normal erythrocyte sedimentation rate (ESR) level

Table 2: Distribution based on their age group with the disorders, admit status and oxygenation status

	Age groups				Total
	21–40	41–60	61–80	81–100	
Disorder					
DM	5	21	61	13	100
HTN	2	8	13	1	24
Admit status					
ICU	57	28	67	13	165
Ward	8	19	9	0	36
SpO_2 (%)					
≤80	39	18	40	8	105
81–90	25	22	33	5	85
91–94	1	3	1	0	5
95–100	0	4	2	0	6
Oxygenation					
Intubation	54	22	60	13	149
NRBM	10	19	14	0	43
Non-intubated	1	6	2	0	9

DM: Diabetes mellites; HTN: Hypertension, ICU: Intensive care unit; NRBM: non-rebreather mask

is around 20 mm/h; however, in this study, 79.1% of patients had an ESR between 36 and 90. Figures 3 and 4 depict the various biomarkers studied.

Total leukocyte count (TLC) between 4000 and 11,000 cells/mm^3 is considered normal; nevertheless, in these situations, 56% of patients exhibited TLC between 25,000 and 75,000 cells/mm^3. The neutrophil-to-leukocyte ratio (NLR) does not have a set standard value for healthy people and is affected by the clinical status of patients. It was claimed that an NLR of more than 9 is in the danger zone; in this study, there was a big peak at 3–5.25 (56%) and a minor peak at 6.75–11.25 (35%). Ferritin is an iron-containing protein, and its test assesses the body's stored iron. It, too, displayed two peaks, 48.25% around 700–1050 and another as a minor 23.38% at 2450–3500. CRP is an acute phase reactant that accompanies inflammation, evaluates inflammation and assesses therapy efficiency. The normal CRP level is undefined. CRP levels of more than 100 mg/L, on the other hand, are termed acute bacterial or viral infection and were found in 77.11% of the patients in this investigation. Table 3 depicts the relationship between biomarkers and the RdRp and E genes based on their admit location. Serum ferritin had a large *P* value in both ICU patients and all patients. Table 4 illustrates the link between clinical symptoms and genes, with no *P* values ≤0.05 indicating an insignificant correlation.

Discussion

Everyone is affected by SARS-CoV-2; however, specific genders, age groups, and those with former conditions are at a greater risk of illness and complications. According to research, although all in the community can catch SARS-CoV-2, particular age, genders or those with previous conditions/immunosuppression are more likely to contract COVID infection than their counterparts.[11-13]

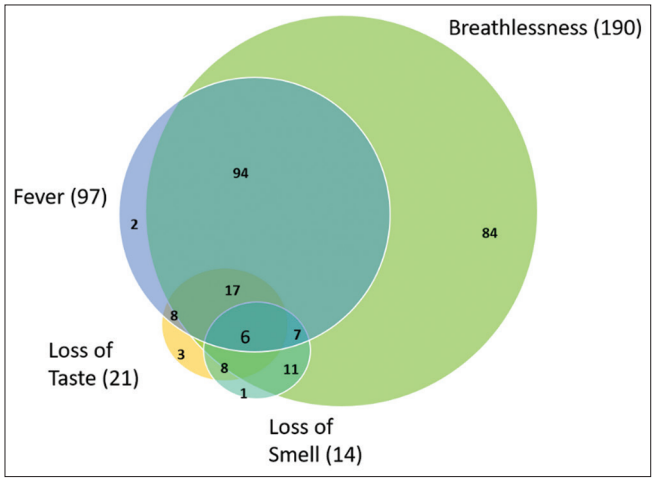

Figure 2: Venn diagram: patients with COVID symptoms of one or more

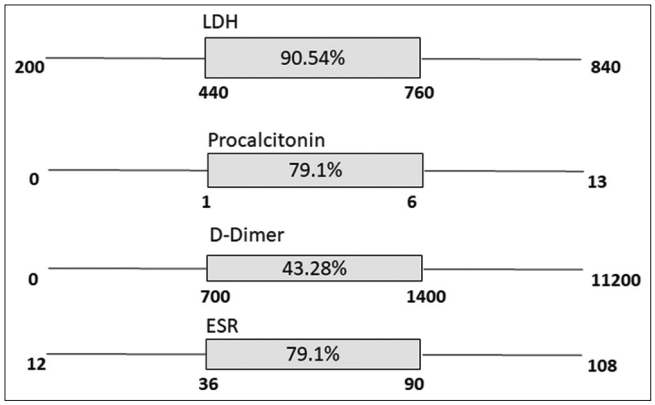

Figure 3: LDH, procalcitonin, D-dimer and ESR levels in COVID patients [LDH: Lactose dehydrogenase; ESR: Erythrocyte sedimentation rate]

The COVID pandemic, despite its widespread distribution, is not fatal enough in comparison to other flu viruses of similar lineage.

We discovered patients aged from 20 to 90 years old among the 201 patients in this study, with 61% being male and 39% being female. Predominantly patients were either middle-aged or elderly. Males made up a majority of the numbers (35.82%), while in old age, males (12.9%) and females (10.45%) were equivalent. In their investigations, Li *et al.* (2020), Khan *et al.* (2020), Chen *et al.* (2020) and Kushwaha *et al.* (2021) discovered a larger prevalence of males with COVID.[13-16] Like our study in an article by Kushwaha *et al.* (2021), most of the male patients fell in the age category of 36–55 years.[13]

Only COVID-positive patients admitted to the hospital were included in the study; patients were classified as follows: Ct ≤ 25 was

Table 3: Correlation between RdRp and E-gene to biomarkers of patients in ICU and Ward (*P*; *rho* value)

Variables	ICU		Ward		Overall	
	RdRp gene	E-gene	RdRp gene	E-gene	RdRp gene	E-gene
ESR						
P	0.6564	0.7798	0.3096	0.3708	0.5185	0.6316
rho	−0.0349	−0.0219	−0.174	−0.154	−0.0458	−0.0340
Procalcitonin						
P	0.3702	0.2381	0.3480	0.3870	0.3722	0.3125
rho	0.0704	0.0926	0.161	0.149	0.0634	0.0718
LDH						
P	0.9943	0.9221	0.7831	0.7623	0.8791	0.8971
rho	−0.00056	0.00767	0.0475	0.0522	−0.0108	−0.0091
CRP						
P	0.7201	0.8143	0.2422	0.1909	0.7816	0.9810
rho	0.0281	0.0184	0.200	0.223	0.0197	0.00169
Ferritin						
P	0.0122	0.0126	0.6175	0.5424	0.0133	0.0102
rho	−0.195	−0.194	0.0861	0.105	−0.174	−0.181
D-dimer						
P	0.1274	0.1026	0.3842	0.5511	0.0747	0.0493
rho	−0.119	−0.128	−0.149	−0.103	−0.126	−0.139
TLC						
P	0.1368	0.2277	0.3273	0.2268	0.2202	0.3576
rho	0.116	0.0944	0.168	0.207	0.0868	0.0652

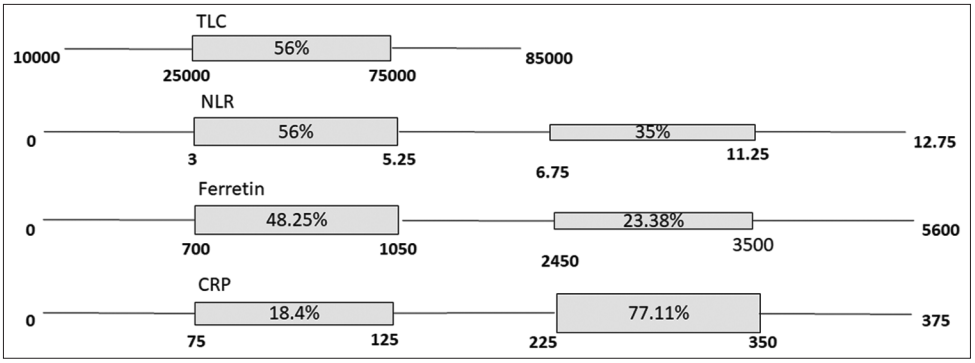

Figure 4: TLC, NLR, Ferritin and CRP levels in COVID patients. [*TLC: total leucocyte count; NLR: neutrophil lymphocyte ratio; CRP: C-reactive protein]

Table 4: Correlation between RdRp and E-gene to symptoms (*P*; *rho* value)

Variable	RdRp gene		E-gene	
	P	*rho*	*P*	*rho*
Fever	0.8302	0.0152	0.9353	−0.00576
Breathlessness	0.6509	−0.0321	0.6956	−0.0278
Loss of smell	0.5681	0.0405	0.3708	0.0635
Loss of taste	0.6179	−0.0354	0.5821	−0.0390

classified as high, Ct ≥ 30 as low and 25 < Ct < 30 as intermediate. According to research by Aranha *et al.*, lower Ct denoted serious COVID,[17] but we found no statistical relationship between Ct, symptomology or admit status. The Ct value of 32–38% of patients was in the intermediate range, whereas 23–28% of patients had a higher Ct value. Likewise, no link was identified between Ct value and patients' symptoms or illness severity in research conducted by Arons *et al.* (2020), He *et al.* (2020), Kimball *et al.* (2020) and Zou *et al.* (2020); the typical Ct in these investigations was 25.5–29.4.[18-21] In research by Huang *et al.* (2020), Liu *et al.* (2021), Schwierzeck *et al.* (2021), Xia *et al.* (2020) and Zheng *et al.* (2020), they found that symptomatic patients had a lower Ct value range between 15.3 and 24.3, and serious patients had an even lower Ct than in comparison to asymptomatic patients with Ct of 28 or higher,[22-26] which contradicts our findings. The efficacy of the Ct value as a prognosis tool is still being debated; an ICMR advice advised against using Ct value as a predictive tool for treating COVID patients.[7]

In our study, we also evaluated comorbid illnesses, with a focus on diabetes and HTN. When the data was analysed, we discovered that 40% of the total patients had just diabetes mellitus, 3% of the total patients had only HTN and 9% of the total patients had both comorbidities. Overall, 52.74% of patients had diabetes, hypertension or both, but when statistical significance was determined, the connection was found to be modest. According to research, people with established diseases are at risk of developing COVID.[27,28] In comparison, studies by Sharif *et al.* (2021), Jin *et al.* (2020), Sanyaolu *et al.* (2020) and Baradaran *et al.*[31] (2020) showed a smaller percentage of patients with HTN and diabetes mellitus.[27-30] In our investigation, we discovered that the number of patients with diabetes was larger than the number of patients with HTN, which may be related to our study having a higher patient count in the ages 41–60 and 61–80 years.

In this study, we observed patients ranging in age from 20 to 90 years old, and we classified them into four age groups with a lower limit of 21 years and upper limit of 100 years and unit of 20 years. Among the various age categories, 61% of the patients (61–80 years old) had diabetes, and 54% of the patients (61–80 years old) had HTN.

We also explored the need for oxygen support and discovered that ages 21–40 years and 61–80 years accounted for 36.2–40.3% of patients needing ventilatory support. The significant percentage of patients requiring mechanical breathing in the aforementioned age group can be linked to critical illness, co-morbidities and immune regression. Comparable figures were identified in the ages 21–40 years; this group's elevated rates can be attributed to them having unpleasant lifestyles. We also analysed patients who needed NIV oxygenation and discovered that the majority were between the ages 41 and 60 years, which might be attributed to the patients in these ages having concomitant diseases that were within tolerable limits.

Patients with COVID-19 arrived at the hospital with a variety of symptoms; we examined four key symptoms that were seen in the individuals included in this research. We discovered that the majority of patients had a mix of symptoms rather than singular ones. According to research, the COVID-19 virus does not just damage the respiratory system, but any organ or organ system that has the ACE-2 receptor; these receptors are abundant in the respiratory system, therefore the respiratory symptoms. In our investigation, we discovered that the majority of patients had both dyspnoea and fever, accounting for 46.8% of the patients, followed by loss of taste with breathlessness accounting for 8.5%. Breathlessness was the most common symptom among patients who arrived with a single symptom, accounting for 41.8% of those investigated. This is consistent with the disease pathophysiology, but this figure cannot be regarded as a genuine value due to a lack of data on positive individuals who were not admitted to the hospital. We examined the statistical relationship between Ct value and patient symptoms but found no meaningful link.[7] Unlike trials such as Jin *et al.* (2020), Khan *et al.* (2020) and Chen *et al.* (2020), we had no patients who came with cough, muscular soreness, or sore throat.[14,16,30] In previous research, a greater number of patients reported fever rather than dyspnoea, but in our study, the majority of patients came with a mix of symptoms.

According to research, people with the condition have lower SpO_2 levels in their blood. This was also seen in this investigation; SpO_2 levels in blood are expressed as a percentage. In our investigation, we discovered the SpO_2 of patients varied between 65 and 97%, with several of them having SpO2 values of 70, 76, 82 and 86, respectively. While the majority of the patients we evaluated required mechanical ventilation, the highest number of patients had oxygen saturation of 82–86%. The statistical significance of the relationship between Ct value and SpO2 levels was equivocal. Borges do Nascimento *et al.*[34] (2020) and Ramirez-Hinojosa *et al.* (2021) noted patients with artificial oxygenation, but the proportion of patients requiring oxygenation was lower, due to the inclusion of non-hospitalized patients, but in this study, 74% required essential mechanized ventilation and 21% obligatory non-invasive oxygen support, and wholly hospitalized patients.[31-33]

Several studies reveal that patients with COVID-19 illness have various abnormal markers. LDH, CRP, procalcitonin, Ferretin, D-dimer and ESR are some examples. These metrics are regarded as biomarkers for determining disease prognostics. In this study, LDH values ranged from 200 to 840 U/L, with 90.5% having

values between 1 and 6 ng/mL, procalcitonin levels ranging from 0.1 to 13 ng/mL and about 79.1% having values between 1 and 6 ng/mL, D-dimer levels ranged from 700 to 11200, but predominantly 700–1400 ng/mL, and Ferretin values ranged from 700 to 5600 ng/mL.

The statistical correlation between biomarkers and Ct value was negligible. When we looked at the connotation between biomarkers and symptoms, we found a modest to moderate but statistically trivial association. Increased levels of biomarkers listed earlier were found in investigations by Borges do Nascimento *et al.* (2020), Shi *et al.* (2020), Khadim *et al.* (2021), and Huan *et al.* (2020).[33-37]

Conclusion

In this observational study, we can note male gender is at an elevated risk of contracting COVID. Also, patients with prior illness undermining or altering their immunity are at an increased risk of COVID infection. Ct value though has a research value, it is a poor prognostic marker when compared to the various biomarkers that have been studied earlier. Among the various biomarkers, procalcitonin is a good indicator for bacterial infection but has minimal or negligible role to play in predicting COVID prognosis. We cannot conclusively state that all our findings are accurate due to a lack of data but further research into the prognostic value of Ct should be conducted which will help in the ongoing scenario.

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Abbreviations: Ct: cycle threshold; CoV: SARS-CoV-2; RT-PCR: reverse transcriptase-polymerase chain reaction; RNA: ribonucleic acid; ICU: intensive care unit; VTM: viral transport medium; E-gene: Envelope gene; RdRp: RNA-dependant RNA polymerase; Ig: immunoglobulin, IL: interleukin; CRP: C-reactive protein; ICMR: Indian Council for Medical Research; LDH: lactose dehydrogenase; CBC: complete blood count; RR: respiratory rate; HTN: hypertension; TLC: total leukocyte count; ESR: erythrocyte sedimentation rate; NLR: neutrophil-to-leukocyte ratio.

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Conflicts of interest

There are no conflicts of interest.

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