# Comorbidities of COVID-19 Patients With Low Cycle Threshold (Ct) Value of Nucleocapsid (N) Gene: An Application to Cluster-Based Logistic Model.

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**Research**

**Keywords:** Comorbidity, COVID-19, rRT-PCR, Ct value; N gene, Cluster-based logistic regression

**Posted Date:** January 19th, 2021

**DOI:** https://doi.org/10.21203/rs.3.rs-147576/v1

# Abstract

**Background:** *Coronavirus* disease 2019 (COVID-19) is a health crisis throughout the world. The widely used Real-time Reverse Transcriptase Polymerase Chain Reaction (rRT-PCR) method is most capable to demonstrate the patient's condition. Comorbidities can make patients more critical.

**Materials and methods:** In this study, we shed light on the low cycle threshold (Ct) value of the N gene in the rRT-PCR test of the COVID-19 patients who had comorbidities and their cure rate as well as the needfulness of ICU (Intensive Care Unit) management. We conducted the research in the Molecular Biology Laboratory of Chittagong Medical College between May and August 2020, then took the telephone interview with 300 positive patients who fulfilled the study criteria. We applied cluster-based logistic regression to analyze the data.

**Results:** Low Ct value of the N gene was found 1.324 times more in Type 2 DM patients and 1.871 times higher in hypertensive patients. Hospitalized patients are 2.480 times more vulnerable to shift in ICU in case of low Ct value of the N gene.

**Conclusion:** While infection with Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) frequently causes severe diseases, suspected cases with comorbid conditions should go through the rRT-PCR as early as possible.


# 1. Introduction

In December 2019, SARS-CoV-2 was first identified which was responsible for the Coronavirus Disease 19 (COVID-19) pandemic [1, 2]. The International Committee on Taxonomy of Viruses (ICTV) declared that the novel coronavirus is officially classified as SARS-CoV-2 in January 2020. They were named like that due to their 9–12 nm long surface spikes that resemble a corona (equal to the crown in Latin). Coronaviruses (CoVs) are enveloped viruses. They are single-stranded and belongs to a positive-sense RNA genome. The SARS-CoV-2 is identified as the 7th coronavirus, becoming the 3rd zoonotic human coronaviruses (HCoV) of the century, and posing serious threats to international health.

It has now affected almost all countries and has led to more than 25 million confirmed cases, with 870497 deaths globally, as of August 2020. There are also asymptomatic or mildly symptomatic cases [1–4], but a huge number of cases progress to severe COVID-19 with high mortality rates. By July 2020, over 600,000 deaths worldwide are recorded. Presently 7 CoVs (including SARS-CoV-2) can cause human respiratory diseases, but to date, only Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV), Middle East Respiratory Syndrome Coronavirus (MERS-CoV), and SARS-CoV-2 have caused a large outbreak with high mortality. They can involve almost all systems of our body such as the respiratory, gastrointestinal, cardiovascular, and nervous systems with variable severity [5].

High viral loads in severe COVID-19 indicates higher viral replication and pathology [6], which is responsible for multiorgan failure [7]. But it is not always true as some studies found asymptomatic

individuals can have high viral loads [8]. In this research, we tried to find out the association of low Ct value in the rRT-PCR method of viral N gene with the comorbidities and clinical outcome of the corresponding patient.

## 2. Materials And Methods

## 2.1. Study design and setting

A cross-sectional study was conducted from May 2020 to August 2020 in the molecular biology laboratory of Chittagong Medical College, Chattogram. Reverse Transcriptase-Polymerase Chain Reaction was done in the QuantStudio 5 Real-Time PCR instrument (96-Well 0.2 ml Block) by Sansure Biotech Novel Coronavirus Nucleic Acid Diagnostic Kit.

## 2.2. Strategy, eligibility criteria and study selection

Patients with a SARS-CoV-2 infection confirmed by an rRT-PCR of the nasopharyngeal and oropharyngeal combined sample within three days of onset of symptoms were included. Presence of symptoms, days of illness, contact with the symptomatic patient, types of personal protective equipment used, traveling history, oxygen requirement, hospital admission history, ICU support, co-morbidities, pH, HCO3, Oxygen saturation, Arterial CO2, Arterial O2, other baseline investigations, and treatment sheet were collected through interviewing over the phone.

The WHO website has provided several Real-time RT-PCR protocols for detecting SARS-CoV-2 in different countries [9]. An automated system was repeated here for the amplification process. 45 cycles were run until the viral complementary DNA (cDNA) could be detected by a fluorescent signal from different channels such as FAM, ROX, and CY5. The presence of the virus in human samples is confirmed by the detection of specific sequences that are unique to SARS-CoV-2 by the rRT-PCR method. The nucleocapsid protein gene N and the RNA-dependent RNA polymerase gene (RdRP) [also reported as Open Reading Frame 1 ab (ORF1ab)] [10] were selected. Internal control was used to check for biases of the total procedure. Positive control such as plasmids containing the complete SARS-CoV-2 N gene [11] was also given. Here, the RNA copy number is expressed by the Ct value of the targeted genes. It is inversely related to the viral load of the corresponding patient.

After getting the results, we listed 300 patients from 436 positive patients for our study; from those, we got relevant history properly. Data collection forms were filled up by clinical characteristics of the patients. Written informed consent was taken from the patients or by next-of-kin if the patient was unable to consent. This study got approval by local regulatory authorities.

## 2.3. Statistical methods

We use descriptive statistics and logistics regression and cluster-based logistics regression to explain the factors involved with the N gene.

# 3. Results

Conditions of the patients are classified into three categories: ICU support with non-invasive procedures, ICU support with mechanical ventilation, and those who need none of these. The patients who had a low Ct value (15.00-30.00) of the N gene, needed more ICU support (13%) than the patients who had a high Ct value (30.01-40.00). In the case of the ORF1ab gene, it was different. In this case, patients with high Ct value used 16.3% ICU (both Non-invasive and Mechanical ventilation) facilities than the patients with low Ct value. In our study, elderly patients (≥ 60 years age) belonging low (15.00-30.00) Ct value of N gene needed the highest (34.6%) ICU support. On the other hand, children did not require any intensive care support. Only 40-59 years age group patients needed mechanical ventilation (8.3%).

From figure 1, it is depicted that, the highest number (79) of cases who were suffering from hypertension (63.7%) had low Ct value (15.00-30.00) of N gene and those patients needed more intensive care support for deteriorating their health conditions than the patients of high Ct value (30.01-40.00). Diabetic patients were in the second (48.4%) position. The patients who had low risk were those who were suffering from CVD (2.4%) with a low Ct value of the N gene. From those comorbid conditions, hypertension and type2 DM are significant with N gene values by chi-square test.

**Table 1.** N gene range with cluster-based comorbid conditions.

| Comorbidity | Categories | Low Ct (15.00-30.00) of N gene | | High Ct (30.01-40.00) of N gene | |
|---|---|---|---|---|---|
| | | Home | Hospital | Home | Hospital |
| Type2 DM | No | 29.00% | 17.00% | 25.00% | 9.00% |
| | Yes | 3.00% | 11.00% | 2.00% | 4.00% |
| Hypertention | No | 25.00% | 16.00% | 25.70% | 7.00% |
| | Yes | 7.00% | 12.00% | 1.30% | 6.00% |

Table 1 represented that the patients who were not suffering from type 2 DM and hypertension, maximum (29.00% and 25.00% respectively) were cured by home treatment, though they had low Ct value of N gene. But, diabetic and hypertensive patients needed more (11.00% and 12.00%, respectively) hospital treatment for those who had low Ct value of the N gene. Besides, having a high Ct value of N gene, non-diabetic and normotensive patients cured more in-home treatment (25.00% and 25.70%, respectively).

**Table 2.** Cluster-based Logistic model: Effect of Comorbidities on low Ct value.

| Logistics Regression model for general data | | | | | | | |
|---|---|---|---|---|---|---|---|
| Comorbidities | Categories | B | S.E. | Sig. | Exp(B) | 95% C.I | |
| | | | | | | Lower | Upper |
| Type 2 DM | Yes | 0.280 | 0.337 | 0.046 | 1.324 | 0.683 | 2.565 |
| | No | — | — | — | 1.000 | — | — |
| Hypertention | Yes | 0.626 | 0.308 | 0.042 | 1.871 | 1.024 | 3.419 |
| | No | — | — | — | 1.000 | — | — |
| Constant | | -1.105 | 0.315 | 0.000 | 0.331 | | |
| Cluster-based Logistics Regression (Home Cluster) | | | | | | | |
| Variable | Categories | B | S.E. | Sig. | Exp(B) | 95% C.I | |
| | | | | | | Lower | Upper |
| Type 2 DM | Yes | 0.175 | 0.603 | 0.071 | 1.192 | 0.365 | 3.888 |
| | No | — | — | — | 1.000 | — | — |
| Hypertention | Yes | -1.718 | 0.582 | 0.003 | 0.179 | 0.057 | 0.562 |
| | No | — | — | — | 1.000 | — | — |
| Constant | | 0.016 | 0.166 | 0.923 | 1.016 | | |
| Cluster-based Logistics Regression (Hospital Cluster) | | | | | | | |
| Variable | Categories | B | S.E. | Sig. | Exp(B) | 95% C.I | |
| | | | | | | Lower | Upper |
| Type 2 DM | Yes | -0.503 | 0.448 | 0.262 | 0.605 | 0.251 | 1.456 |
| | No | — | — | — | 1.000 | — | — |
| Hypertention | Yes | 0.319 | 0.425 | 0.045 | 1.375 | 0.598 | 3.162 |
| | No | — | — | — | 1.000 | — | — |
| Constant | | -0.733 | 0.273 | 0.007 | 0.480 | | |

Building logistics regression and cluster-based logistic regression, we defined the N gene range (low and high) as a dependent variable and significant comorbidities as independent variables. In the logistics regression model for general data, type 2 diabetic patients had a 1.324 times more low Ct value of the N gene. Similarly, in the home cluster, it was 1.192 times higher with CI (0.365, 3.888). But in the hospital cluster, the scenario was reversed (0.605), which was not significant. Hypertensive patients possessed 1.871 times significantly (p = 0.042) more low Ct value of the N gene in the general dataset. Similarly, it

was 1.375 times more in the hospital cluster. On the contrary, the home cluster represented that hypertensive patients had significantly (0.179 times with p-value 0.003) low Ct value of N gene.

**Table 3.** ICU support according to Ct value range of N gene.

| ICU | Low Ct (15.00-30.00) of N gene | | High Ct (30.01-40.00) of N gene | | ICU support type | |
|---|---|---|---|---|---|---|
| | Home | Hospital | Home | Hospital | Non-invasive | Mechanical ventilation |
| No | 30.00% | 17.00% | 25.00% | 5.00% | | |
| Yes | 2.00% | 11.00% | 2.00% | 8.00% | 20.00% | 3.00% |

From table 3, 23.00% of patients needed ICU where 20.00% used non-invasive methods and 3.00% went for mechanical ventilation. Besides, 11.00% of hospitalized patients needed ICU those who had a low Ct value of the N gene. On the other hand, 8.00% of hospitalized patients needed ICU those who had a high Ct value of the N gene.

**Table 4.** Cluster-based Logistic model: effect of N Gene on ICU support.

| Logistics Regression model for general data | | | | | | | |
|---|---|---|---|---|---|---|---|
| Target | Categories | B | S.E. | Sig. | Exp(B) | 95% C.I | |
| | | | | | | Lower | Upper |
| N Gene | Low | -0.187 | 0.278 | 0.052 | 0.830 | 0.481 | 1.430 |
| | High | — | — | — | 1.000 | — | — |
| Constant | | -1.099 | 0.211 | 0.000 | 0.333 | — | — |
| Cluster-based Logistics Regression (Home Cluster) | | | | | | | |
| | Categories | B | S.E. | Sig. | Exp(B) | 95% C.I | |
| | | | | | | Lower | Upper |
| N Gene | Low | -0.182 | 0.598 | 0.076 | 0.833 | 0.258 | 2.691 |
| | High | — | — | — | 1.000 | — | — |
| Constant | | -2.526 | 0.424 | 0.000 | 0.080 | — | — |
| Cluster-based Logistics Regression (Hospital Cluster) | | | | | | | |
| Target | Categories | B | S.E. | Sig. | Exp(B) | 95% C.I | |
| | | | | | | Lower | Upper |
| N Gene | Low | -0.905 | 0.398 | 0.023 | 2.480 | 0.882 | 5.026 |
| | High | — | — | — | 1.000 | — | — |
| Constant | | 0.470 | 0.329 | 0.153 | 1.600 | — | — |

Building logistics regression and cluster-based logistic regression, we defined the condition of the patients into two categories: who needed ICU support and those who did not need any ICU support rolled as a dependent variable. Table 4 represented that ICU patients were 0.17 times lower than those who had a low Ct value (15.00-30.00) of viral N gene in the rRT-PCR method than the high Ct value (30.01-40.00). But, in the case of hospital clusters in the cluster-based logistic regression method, ICU admissions were significantly 2.480 times (CI 0.882, 5.026) higher than those who had a low Ct value of the N gene.

# 4. Discussion

We found that patients with low Ct values were more likely to have Hypertension (n = 79), Type 2 Diabetes Mellitus (n = 60), COPD (n = 33), Bronchial Asthma (n = 15), CKD (n = 9). This relation may be due to decreased oxygenation in the heart and kidney or increased infection. Another study revealed that SARS-CoV-2 directly infects both the heart and kidney [12]. Renal and cardiovascular complications are common in severe COVID-19 [13, 14]. Further studies are recommended to evaluate the relationships between the lower level of Ct value of the N gene and disease severity.

Maximum patients of COVID-19 had mild symptoms [15], but 18–33% of patients required invasive procedures in ICU and 20% of patients died in the hospital in different studies [16–20]. Previous studies reflect that the low Ct value corresponds with the high viral loads. Our study highlights the low level of the Ct value of the N gene for detecting SARS-CoV-2 can deteriorate the condition of the patients.

Viral loads can demonstrate COVID-19 severity [21] as a prognostic biomarker [22], but there is no harmony across the studies [23, 24]. In our analysis, more patients in ICU had low Ct of N gene, which is similar to the study of Magleby et. al. [21]. SARS-CoV-2 infection based on Ct values helps to identify patients who are in a risky zone and who need more intensive care. This may also help to select antiviral agents (eg, Remdesivir) [25].

There were some limitations in our study. We evaluated only the Ct value of a single time sample and thus could not assess viral load dynamics overtime. Our study population size was small and the study period was short.

# 5. Conclusion

We have demonstrated that developing hazardous conditions of the patients depends on the Ct value range of the N gene, even it is worst when there are comorbidities (Type 2 DM and Hypertension). This information will be useful for the selection of patients for COVID-19 who require special management and follow-up at home or hospital. These findings also highlight the critical role of viral load and suggest that Ct values should be reported to assist clinicians in identifying patients at high-risk conditions.

# Abbreviations

SARS-CoV-2 = Severe Acute Respiratory Syndrome Coronavirus 2

COVID-19 = *Coronavirus* disease 2019

Ct = Cycle Threshold

N = Nucleocapsid

rRT-PCR = Real-time Reverse Transcriptase Polymerase Chain Reaction

ICU = Intensive Care Unit

ICTV = International Committee on Taxonomy of Viruses

CoVs = Coronaviruses

HCoV = Human Coronaviruses

SARS-CoV = Severe Acute Respiratory Syndrome Coronavirus

MERS-CoV = Middle East Respiratory Syndrome Coronavirus

cDNA = Complementary DNA

RdRP = RNA-dependent RNA polymerase gene

ORF1ab = Open Reading Frame 1 ab

DM = Diabetes Mellitus

COPD = Chronic Obstructive Pulmonary Disease

CKD = Chronic Kidney Disease

IHD = Ischemic Heart Disease

CVD = Cardio-vascular Disease

# Declarations

### Ethical approval and consent to participate

The study protocol was assessed and approved by the medical ethics committee of the Chittagong Medical College and Hospital, Chattogram.

### Consent for publication

Not applicable


### Funding

Not applicable

### Acknowledgements

We would like to thanks the editors and referees whose constructive criticism led us to develop the presentation and maintain the quality of the paper. We would also like to thanks the Molecular Biology Laboratory of Chittagong Medical College, Chattogram, to give their almost all opportunity for conducting the research.


### Author contributions

SHUVA DAS: Conceptualization; Roles/Writing - original draft

SUJAN RUDRA: Data curation; Statistical analysis

MD. EHSANUL HOQUE: Supervision

ABUL KALAM: Writing - Review & editing

MOHAMMAD ARIFUR RAHMAN: Validation; Visualization

SWAGATA NANDY SHIZUKA: Investigation

TAZRINA RAHMAN: Methodology

All the authors have accepted equal responsibility for the entire content of this submitted manuscript and approved submission.

### Conflict of interest

The authors declare no conflict of interest.

### Availability of data and materials

Upon reasonable request.

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# Figures

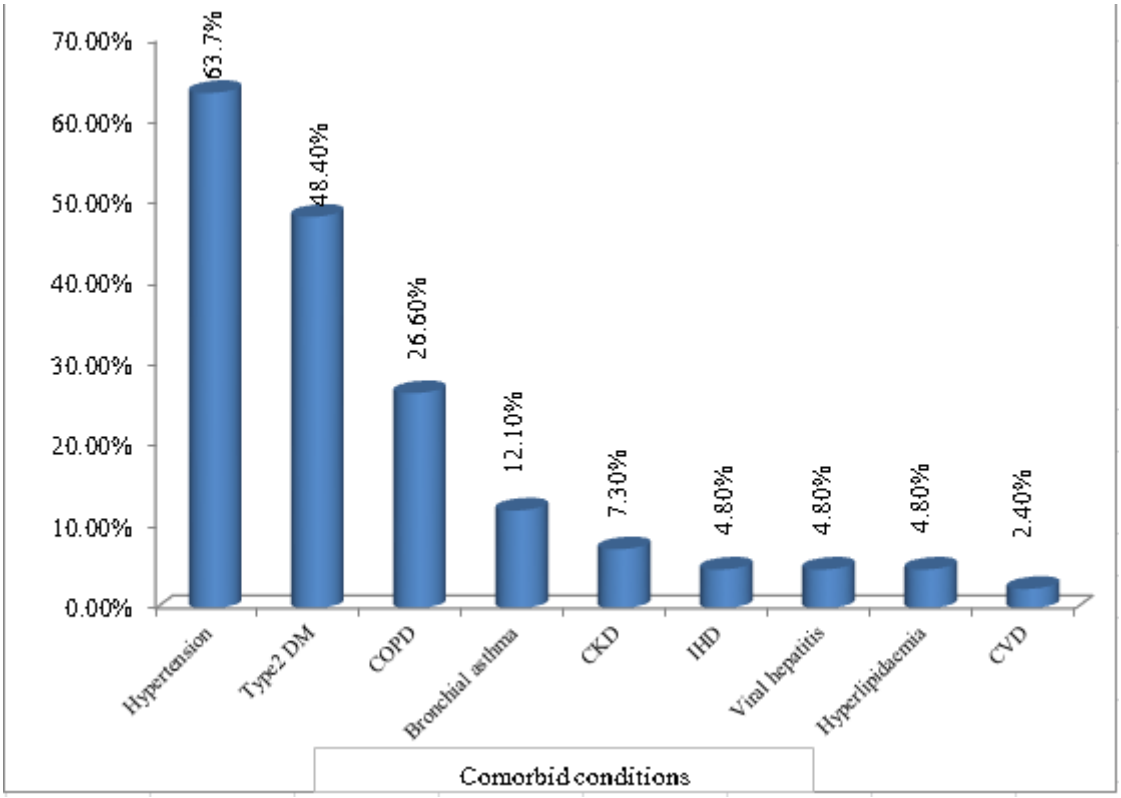


**Figure 1**

Percentage distribution of the patient's comorbid conditions.