Association of Reverse Transcription Polymerase Chain Reaction (RT-PCR) Cycle Threshold (Ct) Value on the Incidence of Cerebrovascular Disease (CVD) in COVID-19 Confirmed Patients of Las Piñas General Hospital and Satellite Trauma Center (LPGH-STC): A Case-Control Study

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Abstract

Objectives: The study aimed to define the cycle threshold (Ct) value of reverse transcription polymerase reaction (RT-PCR) as a potential marker in identifying the risk of COVID-19-confirmed patients in developing cerebrovascular disease (CVD) and to present the risk factors associated with such occurrence.

Methods: The researcher employed a single center, retrospective, chart review, case-control study among adult RT-PCR confirmed, hospitalized COVID-19 patients at Las Piñas General Hospital and Satellite Trauma Center (LPGH-STC) from January to December 2021. The study included 252 patients among 730 RT-PCR-confirmed adult COVID-19 patients who met the study population criteria.

Results: Fifty patients had concomitant CVD, while 202 patients were non-CVD. The majority (n=143, 56.75%) were 19-59 years old male predominance (n=138, 54.76%). More than half of the study population suffered from either hypertension, diabetes, or both, with a high proportion of the subjects being non-compliant or no maintenance medications. Two hundred ten (83.3%) out of 252 patients were unvaccinated. Twenty (7.9%) patients were noted with a history of smoking, and 58 (23%) patients with a history of alcohol intake. The majority of the patients suffered moderate COVID-19 severity during their hospital stay, with a 30.16% (n=76) mortality rate. Ischemic stroke was the most common CVD, with 38 (76%) occurrences. Acute respiratory failure was the leading cause of death, followed by ARDS, brainstem failure, and ACS. The median cycle threshold among CVD patients was 32.84, significantly higher than the majority at 28.64. A higher mean Ct value was noted among patients with CVD infarct at 33.44 as compared to 26.83 among patients with Hemorrhagic Stroke. Utilizing the Point-Biserial Correlation Coefficient to analyze possible association between the Ct value and the occurrence of stroke, a 0.22 correlation coefficient implied a weak positive correlation between the Ct value and CVD occurrence.

Conclusion: The relationship between the cycle threshold (Ct) value and the occurrence of CVD exists weakly, and factors that might affect this relationship must be addressed and resolved. Interpreting Ct value results also requires clinical context; hence, careful utilization of such data must always be observed. Several factors, including old age, male gender, co-existing comorbidities such as hypertension and diabetes mellitus, lack of maintenance medication and non-compliance, vaccination status, smoking, and alcohol intake history, contributed to the poorer outcome of the patients and the high probability of having a stroke.

Keywords: *RT PCR Cycle Threshold, COVID-19, Cerebrovascular disease*

Introduction

COVID-19, being a pandemic, has become a global healthcare burden. No country was spared when the onslaught of disease started plaguing the world's citizenry. Millions of people were put into lockdowns, and the healthcare system has been compromised. According to the statement released by the World Health Organization last July 30, 2020, the COVID-19 pandemic has put some health systems under immense pressure and stretched others beyond their capacity. In some

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countries where attacks on healthcare have been noticed, the COVID-19 pandemic has created hostile environments for healthcare providers who have reported incidents of violence, discrimination, and harassment.[1]

In the Philippines, the health system impacts of the pandemic have been different across municipal income classes and topography, contributed by long-standing symptoms of inequitable resource allocation. Community quarantines alongside transport and border restrictions have universally impacted health service access and delivery, affecting patients requiring specialist care the most. Local health systems reinforced gatekeeping mechanisms for secondary and tertiary care through referral systems and implemented telemedicine services to reduce face-to-face consultation.[2]

During the pandemic's start, the disease's symptomatology was vague, but more studies and observations have been considered. In a study done by Jamora et al., it showed that the incidence of stroke among patients with COVID-19 was 3.4% (n=367). More deaths were also reported among patients with stroke and COVID-19 than those without. In addition, more patients with stroke were admitted to the ICU regardless of the cause.[3] In another study by Bhatia and Srivastava, it showed that among 214 patients, 88 were observed to have some neurological symptoms. Among these patients, CVD was observed in six, with ischemic stroke in five and intracerebral hemorrhage in one patient. They also presented a report wherein, out of 221 patients with SARS-Cov-2 infection, 13 (5.9%) patients developed stroke after a median duration of 10 days of infection.[4] These studies showed that cerebrovascular disease (CVD) in the acute setting may be a telltale sign of an ongoing COVID-19 infection.

In this regard, a direct relationship between stroke and COVID-19 might exist. In a study done by Yu T. et al., they concluded that CVD was a common neurological complication in patients with COVID-19.[5] Moreover, Abou-Ismail et al. also reported an alarming seven-fold increase in large vessel strokes in the < 50-year-old age group in New York City, New York (5 patients in 2 weeks during COVID-19 pandemic compared with 0.7 patients pre-COVID).[6]

The study further presented those previous outbreaks of coronaviruses, including SARS-CoV-1 and Middle-Eastern respiratory syndrome (MERS-CoV), have been associated with increased risk of thrombosis. Similarly, the novel SARS-CoV-2 generates a profoundly prothrombotic milieu, as evidenced by a surge in global reports of arterial, venous, and catheter-related thrombosis. Coagulopathy, in the form of venous and arterial thromboembolism, was emerging as one of the most severe sequelae of the disease and a prognostic of poorer outcomes.[6]

The cycle threshold (Ct) in reverse transcription polymerase chain reaction (RT-PCR) SARS-CoV-2 tests was gaining currency as a potential marker for severe disease in patients with COVID-19 illness.[7] In a study by Rao et al., they reported an association between lower Ct values and worsening disease and progression to severe disease and complications. Examining data from 18 studies, the investigators found significant correlations between Ct values and severe disease or death, as well as Ct and the presence of biochemical and hematological markers.[7,8]

In this study, the researcher aimed to define the utility of the cycle threshold (Ct) value of reverse transcription polymerase reaction (RT-PCR) as a potential marker in identifying the risk of COVID-19-confirmed patients in developing cerebrovascular disease (CVD) and to present the risk factors associated to such occurrence.

Methodology

Study Design and Data Source. The study was a single-center, case-control, retrospective chart review and was implemented only within Las Piñas General Hospital and Satellite Trauma Center. The main source of data was the Health Information Management Section of the institution, which covered the whole year of 2021. RT-PCR Ct values were retrieved from the Molecular Laboratory Section of the hospital.

Study Population. The study included adult RT-PCR-confirmed COVID-19 patients hospitalized at Las Piñas General Hospital and Satellite Trauma Center from January to December 2021.

Inclusion Criteria. Gender preference was not employed; however, gender prevalence of male or female was also measured. The study also included patients aged ≥ 19 years old. All index patients included in the study were RT-PCR-confirmed COVID-19 patients admitted within LPGH-STC. Patients with concomitant diagnosis of Cerebrovascular Disease (CVD), either ischemic stroke, hypertensive intracerebral hemorrhage, aneurysmal subarachnoid hemorrhage, or ruptured cerebral arteriovenous (AV) malformation, transient ischemic attack, were utilized as the experimental group.

Exclusion Criteria. All patients without the diagnosis of COVID-19 confirmed at the time of admission were excluded from the study, as well as COVID-19-confirmed patients verified by other modalities such as Gene Xpert, Rapid antigen testing, among others. Pregnant women and patients with known hypercoagulable conditions (such as those with Malignancy, Myeloproliferative disorders, HIV/AIDS, etc.) were also excluded.

Patients who were using anticoagulants or thrombolytics and compliant with those medications prior to the occurrence of CVD were also excluded.

Sample Size. The sample size was determined through an online sample size calculator, *Raosoft*™. A 95% confidence level with a 5% level of significance was employed. All patients included in the study were hospitalized at LPGH-STC between January 1 and December 31, 2021, with a COVID-19 RT-PCR Confirmed diagnosis on admission.[9]

Sampling Method. A simple random sampling method was used using Microsoft Office Home and Office 2019

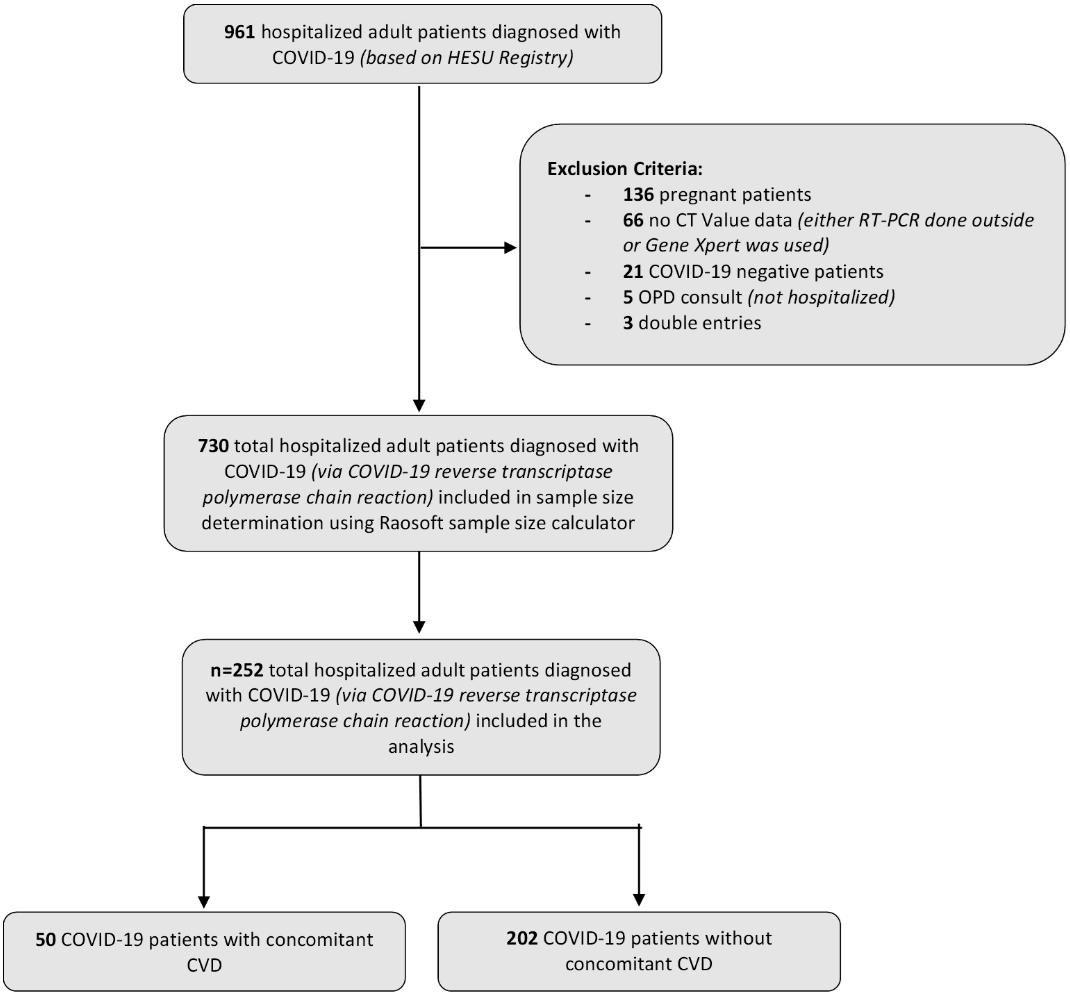

Figure 1. Study Flowchart

Excel's random number generator. The tool was a probability sampling method in which every qualified adult COVID-19 patient admitted at LPGH-STC from January to December 2021 had an equal chance of being chosen.[10]

Variables. Patients' characteristics, such as gender, age, and race, were collected and interpreted. Other baseline characteristics, such as religion and civil status, were not included. Patients' comorbidities, medications taken prior to the CVD event, and compliance with prescribed medications were also collected. The smoking and alcohol consumption status of the patients, as well as the severity of COVID-19 infection, was also analyzed. Cycle threshold (Ct) values in reverse transcription polymerase chain reaction (RT-PCR) were identified at the index date of admission. Causes of death were also tallied and included in the analyses. Incomplete or missing data were not included.

Statistical Methodology. Descriptive statistics such as mean, median, and standard deviation were used to present ratio-scaled variables. The interquartile range was measured in the age variable due to outliers in the data. Frequency and percentage for categorical data were also employed for information on gender, co-morbidities, and CVD types.

The point biserial correlation coefficient was used to measure the association between the cycle threshold (Ct) value and CVD. Comparative analysis was also employed to identify patterns in the data that were important in effectively addressing the research.

Data Source. In this study, the baseline characteristics of the target population, such as age, gender, and COVID-19 vaccination status, were sourced on the front sheet of the patient's charts. Risk factors for CVD and current medications were taken from the admitting physician's history and physical examination form. Cycle threshold (Ct) values in reverse transcription polymerase chain reaction (RT-PCR) were provided by the Molecular Laboratory Department of Las Piñas General Hospital and Satellite Trauma Center. Medical records used for the study were returned to the safekeeping of the hospital's Health Information Management Section (HIMS). All other personal information in the variables mentioned was not included, duplicated, or shared.

Table I. Demographic and Clinical Characteristics of Patients Included in the Analysis

Features	All patients	COVID-19 patients with CVD	COVID-19 patients without CVD
Sample, *n (%)*	252 (100.0)	50 (19.84)	202 (80.16)
Age in years, median *(IQR)*	55.5 (41.5-65.5)	62.5 (51-69)	54 (39-65)
Frequencies of age groups, *n (%)*			
60 years and above	109 (43.25)	27 (54)	82 (40.59)
19-59 years	143 (56.75)	23 (46)	120 (59.41)
Sex, *n (%)*			
Male	138 (54.76)	31 (62)	107 (52.97)
Female	114 (45.24)	19 (38)	95 (47.03)
Nationality, *n (%)*			
Filipino	252 (100.0)	50 (100)	202 (100)
Others	0 (0.0)	0 (0.0)	0 (0.0)
Comorbidities, *n (%)*			
Hypertension	82 (32.54)	28 (56)	54 (26.73)
Diabetes mellitus	23 (9.13)	1 (2)	22 (10.89)
Both Hypertension and DM	40 (15.87)	11 (22)	29 (14.36)
No known comorbidities	107 (42.46)	10 (20)	97 (48.02)
Maintenance medications, *n (%)*			
Compliant	41 (22.16)	5 (10)	36 (17.82)
Non-compliant	15 (8.18)	2 (4)	13 (6.44)
No maintenance medications	196 (77.78)	43 (86)	153 (75.74)
Vaccination status, *n (%)*			
Unvaccinated	210 (83.3)	49 (98)	161 (79.70)
Partially vaccinated	9 (3.6)	0 (0.0)	9 (4.46)
Fully vaccinated	33 (13.1)	1 (2)	32 (15.84)
Smoker, *n (%)*	20 (7.9)	7 (14)	13 (6.44)
Alcoholic beverage drinker, *n (%)*	58 (23)	14 (28)	44 (21.78)
COVID-19 disease severity, *n (%)*			
Asymptomatic	1 (0.4)	0 (0)	1 (0.5)
Mild	47 (18.56)	4 (8)	43 (21.29)
Moderate	77 (30.56)	21 (42)	56 (27.72)
Severe	68 (26.98)	12 (24)	56 (27.72)
Critical	59 (23.41)	13 (26)	46 (22.77)

CVD = cerebrovascular disease, IQR = interquantile range, DM = Diabetes mellitus

Table II. Summary of CVD event on hospitalized adult RT-PCR confirmed COVID-19 patients

Features	Frequency (%)
CVD Event	252 (100)
Ischemic Stroke	38 (15.08)
Hypertensive Intracranial Hemorrhage	8 (3.17)
Subarachnoid Hemorrhage	1 (0.4)
AV Malformation	1 (0.4)
Transient Ischemic Attack	2 (0.8)

Medical records were always reviewed within the premises of HIMS. All attending resident physicians of index patients were not included, gathered, or reproduced. Moreover, all attending nurse/s during the patient's length of stay were not included or reproduced. All copies of ancillaries of each patient were not taken from the patient's chart and were not reproduced, copied, or shared.

Bias. No biases were identified.

Ethical Consideration. No disclosures were identified. The Author declares no conflict of interest. Formal consent was not required for this Retrospective Chart Review (RCR). This RCR contains no studies performed by the author with human participants or animals. All information and data needed for verification were done within the Health Information Management Section of LPGH-STC. This study did not employ photographs, scanning, or other means of duplicating medical records. During the implementation of the study, only one (1) laptop was used to store the data gathered. Medical records were reviewed only within the premises of the medical record section. The medical records used for the study were returned to the HIMS for safekeeping.

Results

Inclusion of patients. The authors identified 961 adult hospitalized patients diagnosed with COVID-19 in LPGH-STC for 2021. Out of this figure, 730 were included in the random sampling after considering the inclusion and exclusion criteria of the study. Using the Raosoft sample size calculator with a 95% confidence level and 5% level of significance, 252 were selected, randomized using Microsoft Excel 2019 random number generator, and were included in the qualitative and quantitative analyses. Fifty patients (19.84%) were identified with

Table III. Comparison of outcomes and causes of mortality in COVID-19 patients with CVD vs. without CVD

Features	All patients (n, %)	COVID-19 patients with CVD (n, %)	COVID-19 patients without CVD (n, %)
Outcome, *n (%)*	252 (100)	50 (19.84)	202 (80.16)
Recovered	176 (69.84)	28 (56)	148 (73.27)
Expired	76 (30.16)	22 (44)	54 (26.73)
Mortality, *n (%)*	76 (100)	22 (28.95)	54 (71.05)
Acute Respiratory Distress Syndrome	19 (25)	3 (13.64)	16 (29.63)
Acute Respiratory Failure	29 (38.16)	6 (27.27)	23 (42.59)
Septic Shock	7 (9.21)	2 (9.09)	5 (9.26)
Multi-organ dysfunction syndrome	1 (1.32)	0 (0.0)	1 (1.85)
Acute Coronary Syndrome	10 (13.16)	1 (4.55)	9 (16.67)
Brainstem Failure	10 (13.16)	10 (45.45)	0 (0.0)

Table IV. Comparison of Cycle Threshold (Ct) Value of COVID-19 Patients with CVD vs. Those Without CVD

Features	All patients n (%)	COVID-19 patients with CVD, n (%)	COVID-19 patients without CVD, n (%)
Sample	252 (100)	50 (19.84)	202 (80.16)
Cycle Threshold (Ct) Value, median *(IQR)*	28.64 (22.13-33.64)	32.84 (28.13-36.02)	27.46 (21.32-32.28)

Table V. Comparison of Mean Ct Value of COVID-19 Patients with CVD Infarct vs. Hemorrhagic Stroke.

Features	Mean Cycle Threshold Value N, (Mean)
CVD Infarct	40 (33.44)
Ischemic Stroke	38 (31.54)
Transient Ischemic Attack	2 (35.44)
Hemorrhagic Stroke, median *(IQR)*	10 (26.83)
Hypertensive Intracerebral Hemorrhage	8 (27.08)
Subarachnoid Hemorrhage	1 (37.60)
Ruptured AV Malformation	1 (14.05)

concomitant CVD, while the remaining 202 (80.16%) did not have any CVD. (See Figure 1)

Demographic and clinical characteristics of included COVID-19 patients. The median age in years of the population was 55.5 years old, with a standard deviation of 16.6. The minimum age among the sample population was 19 years old, while the maximum age was noted at 93 years old. Among those with CVD, the median age was noted at 62.5 years of age, while those without CVD were at 54 years old (*Table I*).

A higher proportion of hospitalized COVID-19 patients was found among those who are non-elderly (19-59 years old) compared to elderly (≥60 years old) (56.75% vs 43.25%). Among those with

CVD in the elderly was slightly higher at 54% than in the age group 19-59, which was 46%, while the contrary was true among those without CVD at 40.59% vs 59.41%).

No significant difference in the proportion of hospitalized adult patients with COVID-19 was found between females and males (45.63% vs 54.37%). The result was reflective of those without

CVD, but a significant difference gender-wise was noted among those with stroke; the male population comprised the majority at 62% compared to 38% of the female population.

All patients involved in the analysis were Filipino in ethnicity. No other nationality was involved in the study.

The majority of the patients were also noted to be unvaccinated with any COVID-19 vaccine at 210 (83.3%), while 33 (13.1%) and 9 (3.6%) were fully and partially immunized, respectively. Only one patient was fully vaccinated among those with CVD, while 32 patients were noted to be without. It was also noted that the majority of patients in both population groups were unvaccinated, while nine patients among those non-CVD patients were partially vaccinated.

Hypertension (HPN) (n=122, 48.4%) and diabetes mellitus (DM) (n=63, 25%) were the most common comorbidities, with only 41 (22.16%) patients compliant with their maintenance medications. At the same time, the rest was either non-compliant (n=15, 8.18%) or no maintenance medications being taken (n=129, 69.73%). Losartan was the most common medication taken by the patients for HPN, while Metformin was used for DM. Only three patients were noted taking Aspirin (ASA) as maintenance medication prior to hospitalization.

Twenty (7.9%) patients were also noted to be smokers in different levels of puff topography; 13 constitute those

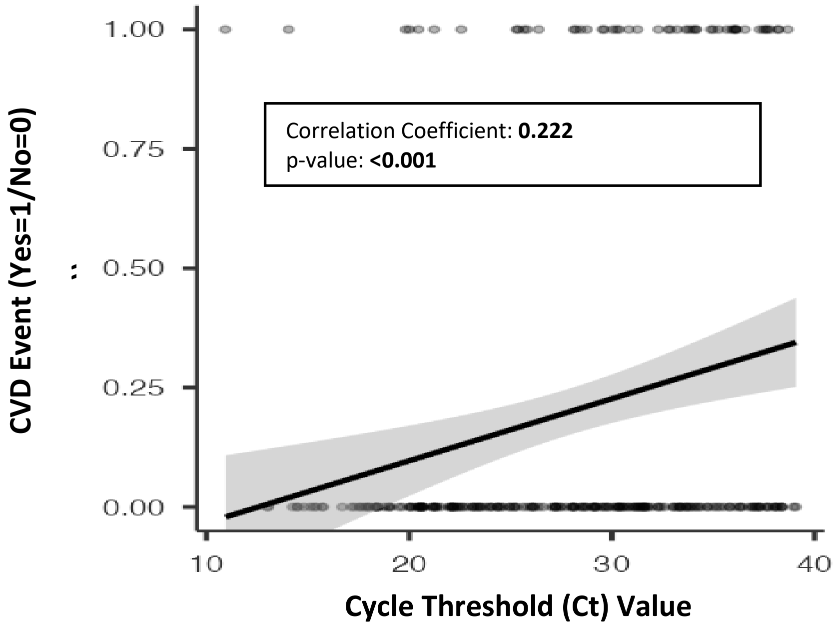

Figure 2. Scatterplot of CVD event vs Cycle threshold value of adult hospitalized COVID-19 patients admitted at Las Piñas General Hospital and Satellite Trauma Center.

non-CVD, while 7 patients are among those with CVD. Fifty-eight (23%) patients drink alcohol on varying levels of alcohol consumption, 44 of which belong to those without CVD while 14 patients to those with concomitant CVD.

Most patients had moderate (n=77, 30.56%) to severe (n=68, 26.98%) COVID-19 infection. In comparison, a significant proportion were noted to have critical (n=59, 23.41%) and mild (n=47, 18.65%) infection. Only one patient (0.4%) was noted to be asymptomatic. Among those with CVD, the majority of the patients had moderate COVID-19 infection at 42%. In contrast, an almost equal proportion of patients were noted with mild, moderate, severe, and critical COVID-19 infection at 21.29%, 27.72%, 27.72%, and 22.77%, respectively, among those without stroke.

Stroke and Outcome. The most common type of CVD was Ischemic Stroke (n=38, 76%), followed by Hypertensive Intracerebral Hemorrhage at 16% (n=8). Only 2 patients had a transient ischemic attack, while one patient had a subarachnoid hemorrhage, and 1 with ruptured arteriovenous malformation. A total of 50 patients (19.84%) constitutes the population with concomitant occurrence of CVD among the study population. In total, 176 (69.84%) patients recovered, while 30.16% (n=76) of the study population expired. Among those with CVD, the recovery rate is slightly higher at 56% (n=28) than those who died (n=22, 44%). The same findings were noted for those without CVD events at 73.27% (n=148) vs. 26.73% (n=54), respectively (*Table II*).

Mortality. A total of 76 patients (30.2%) died among the study population, while 176 patients (69.8%) recovered. Acute Respiratory Failure (ARF) (n=29, 38.16%), acute respiratory distress syndrome (ARDS) (n=19, 25%), Brainstem Failure (n=10, 13.16%), and Acute Coronary Syndrome (ACS) (n=10, 13.16%) were the typical causes of mortality. Among those who died, a significantly higher proportion of patients were found in patients with CVD (1:2) compared to those without (1:3); among those who died due to ARF, ARDS, ACS, and Brainstem Failure, a statistically higher percentage of patients were found in patients with stroke (*Table III*).

Cycle Threshold Value and CVD. The sample population's median cycle threshold value (N Gene) was noted at 28.6 with a standard deviation of 6.85. The minimum Ct value was 10.9, while 39.1 was the maximum. The mean Ct value among those who had CVD Infarction was noted at 33.44, slightly higher than those who had hemorrhagic stroke at 26.83. The patient who suffered from subarachnoid hemorrhage was noted to have the highest Ct value at 37.60, followed by TIA and Ischemic with 35.44 and 31.54, respectively (*Table V*). Using the Point Biserial Correlation analyses to measure the strength of association or co-occurrence between the Ct value and stroke, the correlation coefficient was noted at 0.222 with a p-value of <0.001. The upper 95% class interval was reported at 0.337, while the lower 95% class interval was at 0.102.[11,12]

The point biserial correlation coefficient was used to measure and describe the relationship between the cycle threshold value and the occurrence of

CVD among hospitalized adult RT-PCR-confirmed COVID-19 patients. Analysis showed a 0.222 correlation coefficient equivalent to a weak positive correlation between variables. Correlation coefficients may range between -1 to +1. The closer to 1 (-1 or +1) the coefficient was, the stronger the relationship; the closer to 0 the coefficient was, the weaker the relationship.

A positive correlation means that the two variables tend to change in the same direction; as one increases, the other also tends to increase. However, by applying this finding to our study, one can conclude that an increase in the Ct value *(decrease in the viral load)* increases the tendency for a stroke. In the same perspective, a variable moving from the zero category to the 1 category was associated with an increase in Y, and/or higher Y values tend to co-occur with category 1.

A point biserial correlation coefficient was employed since there were two dichotomous (binary) variables and one continuous variable. Binary variables were variables of nominal scale with only two values. Variables in this study were measured on a nominal level (0,1). Those with an occurrence of CVD were assigned 1, while those without were assigned 0.

The scatterplot diagram showed (see *Figure 2*) the location of each data point formed by a pair of Ct values and the occurrence of CVD. It showed a positive slope and indicates a positive relationship between the patients' Ct value and the occurrence of stroke. (*Table IV*)[12,13]

Discussion

The researcher presented a case-control study involving 252 hospitalized adult RT-PCR-confirmed COVID-19 patients with substantial information on the demographics, clinical features, and neurological characteristics. Furthermore, the researcher investigated the possible association or trends towards association on the COVID-19 patients' reverse transcriptase polymerase chain reaction (RT-PCR) cycle threshold (Ct) value and the occurrence of cerebrovascular disease (CVD).

The increase in the incidence of new-onset cerebrovascular disease (CVD) in the context of COVID-19 infection has been established based on the findings presented by Espiritu, A. et al. (2021) in their Philippine CORONA Study.[14] It was stated that CVD was the second most common neurological disorder/complication at 3.37% among 10,881 patients involved in the trial. Encephalopathy claims the top spot at 5.72%, and seizure events at third place at 1.16%.

Data from the CORONA Study also showed that a significantly higher proportion of COVID-19 patients with new-onset neurological symptoms (NNS) had respiratory failure attributed to central neurological causes compared to those in the non-NNS group. These findings made this study relevant because it can provide healthcare practitioners with a possible prognostic marker to help aid in decision-making on whether to give or not to give a prophylactic medication to prevent the occurrence of stroke.

In this case-control study, the researcher showed that approximately one in every four hospitalized adult COVID-19 RT-PCR-confirmed patients (19.84%) had concomitant CVD during their hospitalization. The median Ct value of this group of patients was noted at 32.84, which was significantly higher than those who did not have a CVD event at 27.46. The mean Ct value of those who had CVD infarction was noted to be higher at 33.44 as compared to 26.83 for those who suffered hemorrhagic stroke.

It should be noted that the RT-PCR test (the gold standard confirmatory test for coronavirus disease 2019) is performed by repeatedly replicating target viral material in the sample (in this study, the N-gene) to the point that it becomes detectable. The number of cycles before the virus is detectable is known as the cycle threshold (Ct). The higher Ct value may indicate a test from someone with minimal detectable viral RNA on their initial swab and may not be infectious or have an ongoing active infection. On the other hand, the fewer cycles required means a greater concentration of viral genetic material in the original sample.

Correlating the high Ct value of patients with CVD to the natural history of COVID-19 infection, one can infer that this group of patients may be in the hyperinflammatory phase of COVID-19 infection at the time of hospital admission. In this phase, the host's inflammatory response is high, with the disease severity increasing due to multiple factors, including cytokine storms. SARS-CoV-2, the causative agent of COVID-19, attacks the immune system, causing an exaggerated and uncontrolled release of pro-inflammatory mediators. Recent studies propose an active role of coagulation disorders in disease progression. This hypercoagulability has been displayed by a marked increase in D-Dimer in hospitalized patients.

The data presented, and the statistical analysis revealed that the occurrence of CVD and the patients' Ct value at the time of admission have a weak positive correlation, with a correlation coefficient of 0.222. The term correlation is synonymous with relationship. However, the fact that there was a relationship between two variables does not mean that changes in one variable can cause changes in the other variable.

Many reports have suggested age and comorbidities like hypertension and diabetes to be associated with higher mortality in COVID-19. Based on the data of patients with COVID-19 and stroke, seven (14%) patients had a history of smoking, and 14 (28%) patients had a history of alcohol intake. One (2%) patient has diabetes, 28 (56%) patients are hypertensive, and 11 (22%) are both diabetic and hypertensive. Moreover, patients with stroke were more likely to have other underlying disorders, including hypertension and diabetes mellitus.[15]

Age group 19-59 years old was also noted to be more susceptible to be infected with COVID-19 than to those 60 years and above. It is more likely attributed to the strict lockdown rules implemented by the government during this time amongst the elderly, immunocompromised, children, and other vulnerable population groups.

Incidence of hypertension and diabetes mellitus among the study population was also accounted for since these conditions present a higher risk of occurrence of CVD, as stated by Yu, T. et al. (2021) in their study SARS-CoV-2-Associated Cerebrovascular Disease Amid the COVID-19 Pandemic: A Systematic Review. Results were congruent with their findings as the data revealed that a higher proportion of patients with CVD also have co-existing hypertension (56%), DM (2%), and both HPN and DM (22%). Moreover, lack of maintenance medications and non-compliance to prescribed medications for HPN and DM was also seen as a contributing factor to the patient's outcomes. These can be attributed to poor access to healthcare and poor follow-up rates exacerbated by strict lockdowns, a lack of reliable network connections to telemedicine, and a lack of funds due to widespread lay-offs and economic opportunities.

The vaccination rate was also very poor, with 83.3% of the study population being unvaccinated. This may be due to hesitancy, misconceptions, and related fears and anxiety of the populace towards COVID-19 vaccines, as well as poor access to these vaccines, as supply was not that high during this time.

Ischemic stroke comprised 76% *(n=38)* of the CVD among the study population. Only eight (16%) patients were noted as having hypertensive intracranial hemorrhage, 2 (4%) patients with transient ischemic attacks, and one (2%) patient each for subarachnoid hemorrhage and AV malformation. The occurrence of stroke is multifactorial, with age, lifestyle, comorbidities, and compliance to maintenance medications, among others, considered pertinent. However, in this study, considering a higher Ct value that coincides with the hyperinflammatory phase of COVID-19, it can be determined that high cytokine activity brings the possibility of immune disarray and a potential role of immunomodulation. This intense cytokine activity could potentially induce heightened inflammation, dysregulation of thrombotic balance, and vascular inflammation in already existing plaques in the vessel wall. However, this needs to be proven with appropriate evidence.

Conclusion

The relationship between the cycle threshold (Ct) value and the occurrence of CVD exists weakly, and factors that might affect this relationship must be addressed and resolved. Interpreting Ct value results also requires clinical context; hence, careful utilization of such data must always be observed.

Several factors, including old age, male gender, co-existing comorbidities such as hypertension and diabetes mellitus, lack of maintenance medication and non-compliance, vaccination status, smoking, and alcohol intake history, contributed to the poorer outcome of the patients and the high probability of having a stroke.

Further studies should be made to establish the Ct value as a prognostic factor in the possible occurrence of stroke among COVID-19-infected patients.

Recommendation

A lot of controversies arise with the utilization of cycle threshold value as a possible marker in prognosticating the potential outcome of COVID-19 infection. One of these is the clinical scenario that can result in a positive test with a high Ct value in someone who may still be or may soon become infectious. It may be worthwhile to conduct a prospective study documenting a series of RT-PCR Ct values of an adult hospitalized COVID-19-confirmed patient with concomitant CVD to shed light on this issue.

Impairment caused by stroke to all COVID-19 patients with concomitant CVD must be objectively measured also utilizing a standard tool such as the National Institutes of Health Stroke Scale (NIHSS) to explore the possible correlation of stroke severity to the Ct value level and disease outcome.

Ct value can also be correlated with the viral load, pre-analytic, analytic, and post-analytical variables such as collection, technique, specimen type, sampling time, viral kinetics, transport and storage condition, nucleic acid extraction, viral RNA load, primer designing, real-time PCR efficiency, and Ct value determination method. A study that will standardize such methods will likewise be helpful.

A study investigating the relationship between inflammatory markers such as D-Dimer and the Ct value among COVID-19 patients in the occurrence of stroke is also useful in establishing the pathophysiology and the natural history of the disease.

References

1. World Health Organization. (2020, July 30). *Attacks on health care in the context of COVID-19*. https://www.who.int/news-room/feature-stories/detail/attacks-on-health-care-in-the-context-of-covid-19
2. Bayani, D. B. and Tan, S. G. (2021, March). Health Systems Impact of COVID-19 in the Philippines. *Center for Global Development (CGD) Working Paper 569.* Washington, DC: Center for Global Development. https://www.cgdev.org/publication/health-systems-impact-covid-19-philippines.
3. Jamora, R. D., et al. (2022). Incidence and risk factors for stroke in patients with COVID-19 in the Philippines: An analysis of 10,881 cases. *Journal of Stroke and Cerebrovascular Disease, Vol. 31.* Elsevier Inc. https://doi.org/10.1016/j.jstrokecerebrovasdis.2022.106776
4. Bhatia, R. and Srivastava, P. (2020, June). COVID-19 and Stroke: Incidental, Triggered or Causative. *Annals of Indian Academy of Neurology, Vol. 23, Issue 3.* Wolters Kluwer – Medknow. DOI: 10.4103/aian.AIAN_380_20
5. Yu, T., et al. (2021, November). SARS-CoV-2-Associated Cerebrovascular Disease Amid the COVID-19 Pandemic: A Systematic Review. *Infection and Drug Resistance 2021.* Dove Medical Press Limited. https://doi.org/10.2147/IDR.S340314

6. Abou-Ismail, M. Y., et al. (2020, June). The hypercoagulable state in COVID-19: Incidence, pathophysiology, and management. *Thrombosis Research 194 (2020) 101-115.* Elsevier Ltd. https://doi.org/10.1016/j.thromres.2020.06.029
7. American Association of Clinical Chemistry. (2020, December 3). *SARS-CoV-2 Cycle Threshold: A Metric That Matters (or not)*. https://www.aacc.org/cln/cln-stat/2020/december/3/sars-cov-2-cycle-threshold-a-metric-that-matters-or-not
8. Rao, S. et al. (2020, July 28). A Systemic Review of the Clinical Utility of Cycle Threshold Values in the Context of COVID-19. *Infect Dis Ther.* https://doi.org/10.6084/m9.figshare.12668408
9. Sample Size Calculator by Raosoft, Inc. (2004). *Raosoft, Inc.* [Internet Website]. Retrieved from http://www.raosoft.com/samplesize.html
10. Donnelly, Jr., R. A. (2007). *The Complete Idiot's Guide to Statistics (2nd ed.).* New York, USA: Penguin Group (USA).
11. Cohen, L. (1998). Statistical Power Analysis for the Behavioral Sciences (2nd ed.). Hillsdale, NJ: Lawrence Erlbaum Associates, Publishers.
12. The Jamovi Project (2022). *Jamovi.* (Version 2.3) [Computer Software]. Retrieved from https://www.jamovi.org.
13. R Core Team (2021). *R: A Language and environment for statistical computing.* (Version 4.1) [Computer software]. Retrieved from https://cran.r-project.org. (R packages retrieved from MRAN snapshot 2022-01-01).
14. Espiritu. A., et al. (2021). The Philippine COVID-19 Outcomes: A Retrospective Study of Neurological Manifestations and Associated Symptoms (The Philippine CORONA Study): A Protocol Study. *BMJ Open 2020.* Retrieved from: https://bmjopen.bmj.com/content/bmjopen/10/11/e040944.full.pdf
15. Li Y, Wang M, Zhou Y, et al. Acute Cerebrovascular Disease Following COVID-19: A Single Center, Retrospective, Observational Study (3/3/2020). 2020. Available from: http://dx.doi.org/10.2139/ SSRN.3550025. Retrieved from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8074652/