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ORIGINAL ARTICLE

THE ASSOCIATION OF RT-PCR CYCLE THRESHOLD VALUE WITH TIMING OF SAMPLE COLLECTION AND PRESENTING MANIFESTATIONS OF COVID-19 AMONG PEDIATRIC PATIENTS ADMITTED IN A TERTIARY HOSPITAL IN DAVAO CITY

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ABSTRACT

Background: Cycle threshold (Ct) value has gained clinical attention in the study of COVID-19 as it has been used as an indirect method of quantifying the viral RNA in a specimen. Investigating its utility will aid in discovering transmission dynamics and clinical decision-making.

Objective: To determine the association between the RT-PCR Ct value with the timing of sample collection and presenting manifestations of COVID-19 among pediatric patients.

Methods: A retrospective, analytical, cross sectional research was done which included all patients with a positive RT-PCR admitted in a private tertiary hospital in Davao City. Ct values and clinical and demographic data were retrieved. Descriptive statistics were used to present and analyze the profiles. Independent t-test, analysis of variance and Pearson r were used for comparison and correlation of variables.

Results: A total of 48 subjects were included in the study. Of the presenting manifestations, the most common were fever and cough. There was no significant difference in the Ct values of the different presenting manifestations.

There was a weak positive correlation between Ct value and time of sample collection (r 0.3027). A weak negative correlation between Ct value and number of presenting manifestations was also noted (r -0.2045).

Conclusion: Ct value increases with time and is significantly low during the first three days of illness. There is no significant difference in the mean Ct values of the different presenting manifestations. Furthermore, the higher number of presenting manifestations is associated with lower Ct values.

KEYWORDS: *COVID-19, Cycle threshold value, correlation*

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The author declares that the data presented are original material and has not been previously published, accepted or considered for publication elsewhere; that the manuscript has been approved by the author, and that the author has met the requirements for authorship.

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INTRODUCTION

The coronavirus disease 2019 (COVID-19) was declared by the World Health Organization as a pandemic in March 2020.[1,2] Current data on its incidence and prevalence in children appear to be an underestimation but the numbers have been steadily increasing.[1-4] The gold standard in diagnosing COVID-19 is real-time reverse transcription polymerase chain reaction (RT-PCR) from nasopharyngeal and oropharyngeal swabs. It confirms infection by creating many copies of the same genetic material from the virus in a process called amplification. The number of amplification cycles required for the viral RNA to reach a detectable level is called the cycle threshold (Ct) value. The test reports as negative or positive using a specific Ct value per machine.[4,5]

Ct value has gained clinical significance in the study of COVID-19 as it has been used as an indirect method of quantifying the viral RNA in a specimen . Studies showed that a lower Ct value signifies a higher viral load and vice versa.[4-7] Multiple studies have attempted to correlate Ct values with the type and number of symptoms, as well as determine its temporal trend but the data available show conflicting results, and the use of the Ct value remains ambiguous. [4, 6-7, 9-10]

Although cases of COVID-19 comprise a smaller percentage in children, they remain vulnerable.[1,8] School attendance, performance and learning are greatly affected as most of the children are unvaccinated, hence longer days of isolation are required. Even though most of the cases in children are mild, parents and guardians remain anxious and fearful when faced with the diagnosis. As community health restrictions have been lifted and face-to-face classes have resumed, investigating the use of Ct value as a surrogate for viral load and infectiousness may help pave the way for faster and more efficient clinical decision-making. Our study findings may contribute in reducing disease transmission and, ultimately, morbidity, mortality, and economic burden.

This study intended to determine the association between the RT-PCR Ct value with the timing of sample collection and presenting manifestations of COVID-19 among pediatric patients admitted in a tertiary hospital in Davao City, Philippines. Specifically, to determine the demographic and clinical profile of the patients, to determine and compare the correlation of the mean Ct values in relation to timing of sample collection, to compare Ct values among the different presenting manifestations, and lastly to know if there is a correlation of Ct values with the number of presenting manifestations.

MATERIALS AND METHODS

Study Design

This is a retrospective, analytical, cross-sectional research study.

Study Setting and Population

The study was conducted in a private tertiary hospital in Davao City, Philippines.

Inclusion Criteria

All pediatric patients admitted from May 2021 to February 2023 with a positive SARS-CoV-2 RT-PCR done in the study setting, with a Ct value of 40 and below and without a prior COVID-19 infection were included in this study.

Exclusion Criteria

Children with co-morbidities were excluded from the study so as to reduce bias as presenting manifestations will be correlated with Ct values.

Sampling Design

This study utilized total enumeration of all subjects who qualified the inclusion and exclusion criteria.

Sample Size

To determine the minimum sample size for this study, the following assumptions and the sample size

calculation for proportions found in Open Source Epidemiologic Statistics for Public Health (OpenEpi) were used.

1. The confidence limit was set at 90%.
2. The assumed percentage of patients with respiratory and non-respiratory manifestations was at least 50%, the default in our calculation.

The minimum sample size for this study is 47.

Data collection and procedure

Using the medical records log of admissions, all pediatric patients who satisfied the study criteria were included in the study. Using a data collection form, demographic features such as age and sex, clinical features such as time of symptom onset to the positive test, and presenting manifestations during the testing time were retrieved from the patients' charts. The patients' RT-PCR cycle threshold (Ct) values were retrieved from the Molecular Laboratory. Using the Sansure RT-PCR assay, a maximum of 40 thermal cycles was set as the cut-off for determining a positive or negative result. Using the machine's specific cut off value of 40, there were no indeterminate results.

Each patient's presenting manifestations were identified and categorized as respiratory, gastrointestinal, other manifestations (non-respiratory and non-gastrointestinal), combination of respiratory and gastrointestinal, combination of respiratory and other manifestations, combination of gastrointestinal and other manifestations, combination of respiratory, gastrointestinal and other manifestations. Patients were then grouped as to the number of presenting manifestations they presented, and the mean Ct values per group were determined.

Statistical analyses

The clinical and demographic profiles were analyzed using frequency and percentage. Ct values of patients were averaged per category of presenting manifestation and per number of presenting manifestations. Independent t-test and analysis of variance were used to compare the variables, while Pearson correlation r was used to determine the relation between the variables. All statistical tests used a 0.05 level of significance.

Ethical Considerations

The researcher has Good Clinical Practice (GCP) certification and sought approval from the Institution's Ethics and Review Board before conducting the research. Informed consent was waived as this study involved analysis of existing data and posed no risk to participants. All charts and SARS-CoV-2 RT-PCR results were accessed through the Medical Records Section and Molecular Laboratory with permission from the Medical Director and the Chairman of the Department of Pathology and Laboratory.

To maintain the patients' anonymity, the data collected were coded. All information obtained will be treated as confidential and will be stored on the researcher's local drive for a duration of five years following the completion of data collection. There are no conflicts of interest to declare. There was no grant received from commercial sectors or agencies.

Definition of Terms

Reverse Transcription Polymerase Chain Reaction (RT-PCR) – a test for the qualitative detection of nucleic acid from SARS-CoV-2 in respiratory specimens collected from individuals suspected of COVID-19; is a molecular test that detects SARS-CoV-2 and confirms infection by creating many copies of the same genetic material from the virus in a process called amplification; reports as negative or positive using a specific threshold called cycle threshold value [1, 3-4, 12]

Cycle Threshold value (Ct value) – indicates how many times a machine needed to try to copy a particular virus's genetic material before being able to detect that material on Polymerase Chain Reaction (PCR) test [11-12]; uses the Sansure RT-PCR assay with a cut off of 40 thermal cycles in indicating a positive or

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negative result; the official result of the RT-PCR is written in numerical value and interpreted as follows:

Positive RT-PCR result - a patient who has a Ct value of 40 and below

Negative RT-PCR result - a patient who has a Ct value of more than 40

Day of illness – the number of days counted from the day of onset of signs and symptoms attributed to COVID-19

Timing of sample collection – the day of illness when the sample for RT-PCR was collected

Presenting manifestation –the initial signs and symptoms presented by the patient at the time of RT-PCR testing

Respiratory manifestations – a patient who presents with any sign and/or symptom attributable to the respiratory system such as sore throat, pharyngeal erythema, stridor, nasal congestion, rhinorrhea, cough, dyspnea, fast-breathing, chest-indrawing and/or oxygen desaturation

Gastrointestinal manifestations – a patient who presents with any sign and/or symptom attributable to the gastrointestinal system such as decrease or lack of appetite, vomiting, diarrhea and/or abdominal pain

Other manifestations – a patient who presents with any sign and/or symptom not attributable to the respiratory or gastrointestinal system, such as fever, headache, rash, body malaise, decreased activity, and/or seizure

RESULTS

A total of 48 subjects were included in this study. Table 1 summarizes the demographic and clinical profile of the patients. Majority of the subjects, 17 (35%), belonged to the 1-3 years old age group and there was a male preponderance of 32 (67%).

Forty-one (85%) were classified as mild COVID 19, seven (15%) were moderate and none of the subjects were severe or critical.

Table 1. Demographic and Clinical Profile of Pediatric Patients with Positive RT-PCR

Characteristics	N = 48
Age	
0-12 months	3 (6%)
1-3 years old	17 (35%)
4-5 years old	5 (10%)
6-11 years old	9 (19%)
12-18 years old	14 (29%)
Sex	
Male	32 (67%)
Female	16 (33%)
Severity Classification	
Mild	41 (85%)
Moderate	7 (15%)

Table 2 shows the number and percentage of the subjects' presenting manifestations. The most common among them were fever with a total of 37 (77%) and cough with 19 (40%).

Table 2. Presenting Manifestations of Pediatric Patients with Positive SARS-CoV-2 RT-PCR

Characteristics	N = 48
Respiratory	
anosmia	1 (2%)
coryza	10 (21%)
cough	19 (40%)
dyspnea	3 (6%)
fast-breathing	1 (2%)
hoarseness of voice	2 (4%)
odynophagia	1 (2%)
sore throat	4 (8%)
stridor	2 (4%)
Gastrointestinal	
abdominal pain	10 (21%)
ageusia	1 (2%)
decrease in appetite	10 (21%)
diarrhea	3 (6%)
nausea	1 (2%)
vomiting	17 (35%)
Other manifestations	
body malaise	5 (10%)
decreased activity	6 (16%)
fever	37 (77%)
headache	7 (15%)
rash	2 (4%)
seizure	2 (4%)

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Table 3 shows the number and percentage of the subjects' presenting manifestations categorized into respiratory, gastrointestinal and other manifestations. The most common among them were the combination of respiratory and other manifestations and the combination of gastrointestinal and other manifestations with a total of 14 (29%) for each category. No patient presented with a combination of respiratory and gastrointestinal manifestations.

Table 3. Presenting Manifestations of Pediatric Patients with Positive SARS-CoV-2 RT-PCR based on Symptom Categories

Category	N = 48
Respiratory	3 (6%)
Gastrointestinal	2 (4%)
Other manifestations	6 (13%)
Respiratory and gastrointestinal	0 (0%)
Respiratory and other manifestations	14 (29%)
Gastrointestinal and other manifestations	14 (29%)
Respiratory, gastrointestinal and other manifestations	9 (19%)

As shown in table 4, the mean Ct value of the subjects was 28.05 ± 8.83. Of the 48 subjects, 11 patients had a Ct value of 20 and below.

Table 4. Cycle Threshold Values of Pediatric Patients with Positive SARS-CoV-2 RT-PCR

Ct Values	Values
Mean ± SD	28.05 ± 8.83
20 and below (14.5 – 19.8)	11 (23%)
20.1 to 30 (20.5 – 28.4)	18 (37%)
30.1 and above (32.7 – 40)	19 (40%)

Table 5 shows the mean Ct values in terms of timing of sample collection. It can be noted that 10 (21%) of the subjects underwent testing on day 6 and onwards. The lowest result can be noted on day 3 of illness (22.42 ± 4.98), while the highest result can be noted on day 5 of illness (33.7 ± 7.45). A p-value of 0.039 shows that there is a significant difference in the Ct values collected from each day of illness.

Table 5. Cycle Threshold Value and Timing of Sample Collection among Pediatric Patients with Confirmed COVID-19

Day of illness	N=48	Ct Value	p-value
Day 1	6 (12%)	26.1 ± 4.07	0.039
Day 2	9 (19%)	24.29 ± 5.67	
Day 3	8 (17%)	22.42 ± 4.98	
Day 4	7 (14%)	31.86 ± 6.78	
Day 5	8 (17%)	33.7 ± 7.45	
Day 6 or more	10 (21)	29.93 ± 7.87	

Significant p-value <0.05

Table 6 shows the correlation between the timing of sample collection and Ct values. The Pearson r value is 0.3027 which can be interpreted as a weak positive correlation. This means that Ct value increases concurrently with time but not in a strong or consistent manner. A p-value of 0.0112 shows a significant relationship between the two variables.

Table 6. Correlation between Cycle Threshold Value and Timing of Sample Collection among Pediatric Patients with Confirmed COVID-19

Correlation	Pearson r	p-value
Timing of Sample Collection and Ct Values	0.3027	0.0112

Significant p-value <0.05

Table 7 shows the mean Ct value of each category of presenting manifestations. The lowest result can be noted in the gastrointestinal group (23.90 ± 2.80); however, a p-value of 0.0679 shows that there is no significant difference in the values among the different presenting manifestations.

Table 7. Cycle Threshold Value and Presenting Manifestations of Pediatric Patients with Confirmed COVID-19

Category	N=48	Ct Value	p-value
Respiratory	3	23.93 ± 5.78	0.0679
Gastrointestinal	2	23.90 ± 2.80	
Other manifestations	6	36.29 ± 2.18	
Respiratory and other manifestations	14	24.09 ± 1.86	
Gastrointestinal and other manifestations	14	30.10 ± 2.68	
Respiratory, gastrointestinal and other manifestations	9	27.81 ± 2.89	

The table below shows the correlation between the number of presenting manifestations and Ct values. It can be noted that 16 (33%) had three manifestations at the time of RT-PCR testing. The

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lowest mean Ct value is noted with subjects who presented with five or more presenting manifestations. The Pearson r value is -0.2045 which can be interpreted as a weak negative correlation. This means that to a small degree, Ct values decrease as the number of presenting manifestations increase since the p-value is significant at 0.012.

Table 8. Correlation between Cycle Threshold Value and Number of Presenting Manifestations among Pediatric Patients with Confirmed COVID-19

Number of Presenting Manifestations	N = 48	Ct Values	Pearson r	p-value
1	4 (8%)	27.7 ± 3.4		
2	11 (23%)	27.14 ± 2.98		
3	16 (33%)	29.49 ± 2.43	-0.2045	0.012
4	10 (20%)	28.55 ± 2.1		
5 and more	7 (16%)	22.6 ± 1.3		

Significant p-value <0.05

DISCUSSION

In this study, COVID-19 was more common among males and children one to three years old. In terms of epidemiology, general data state that children of all ages can be infected with SARS-CoV-2, and there is no gender predilection.[1,3-4] In this context, parents and caregivers should be equally cautious in protecting all children, as anyone can get infected. Most of the subjects in this study were diagnosed as mild (85%). This finding is consistent with the literature review. [1,3-5]

The most common presenting manifestations in this study were fever and cough, which is consistent across all other reports.[2-11] The symptoms category of gastrointestinal manifestations yielded the lowest mean Ct value but there was no significant difference in the mean Ct values among the groups of presenting manifestations. This is comparable to the study of Ghamarchehreh, et al which showed no significant difference between the patients with and without respiratory symptoms, but found a significant difference between subjects who presented with diarrhea compared to those who did not.[9] However, a contrasting result was noted in the study of Salvatore et al. and Roversi et al. showing a significantly lower mean Ct value in those with respiratory symptoms.[6, 13] Our study population only had three subjects who presented with diarrhea hence the symptom could not be adequately represented and analyzed.

Ct value is positively correlated with time of sample collection. Our results showed a weak positive correlation between the two variables. This means that Ct value increases concurrently with time but not in a strong or consistent manner. A decreasing trend in the mean Ct values is observed on the first three days of illness, with a sudden rise on the fourth day. This is similar with the study by Romero-Alvarez, et al which also showed increasing Ct values in time, but this study had a relatively small sample size.[5] On the contrary, Ghamarchehreh et al. have reported no significant difference between Ct values and time from symptom onset, however in their study, 80% of the subjects underwent testing on day three and onwards of illness.[9] Meanwhile, the study by Gentile, et al. showed that significantly lower Ct values were noted on the first four days of illness in symptomatic children less than two years of age.[7] In our study, a significant rise in the titer was noted to start on day four of illness. The age group and number of symptoms in the findings of Gentile, et al can probably explain the contradiction with our study. Furthermore, although there was a notable decline in the value on day six and onwards of illness, the value was still significantly higher compared to the values on the first three days of illness. The inconsistency in the rise of the values on day six and onwards can probably explain the weak positive correlation in our study. Still, the relationship was significant. With Ct value as an indirect measure of viral load, this study implies that pediatric patients with COVID-19 have the highest viral load during the first three days of illness. Our study therefore suggests that when children are symptomatic, SARS-CoV-2 RT-PCR testing can be best done on the first three days of illness, and home isolation would be most prudent unless COVID is ruled out.

There is an inverse correlation between Ct value and the number of presenting manifestations. This is contrary to the study by Ghamarchehreh, et al and Al-Shareef, et al which showed that Ct values were not related to the number of symptoms.[9,10] Furthermore, a study by Costa et al. showed no significant difference in the Ct values of symptomatic and asymptomatic children.[14] The first two studies had subjects who represented the entire spectrum of COVID of which the more severe classifications largely affected the number of manifestations the subjects presented, while the study by Costa et al. analyzed Ct values regardless of timing of sample collection. [9, 10, 14] Existing literature suggests that viral load starts to rise around the time before symptom onset. [1, 3] In the study by Costa et al., there was no data as to whether the asymptomatic subjects were presymptomatic or developed symptoms after the testing. This may have contributed to the difference in the results of our study. On the other hand, a community-based cross-sectional study by Chung et al. which analyzed Ct value results of 123 children suggests that symptomatic individuals have significantly lower Ct values compared to asymptomatic ones.[15] This further supports the findings in our study. Nevertheless, our results suggests that testing and isolation are highly recommended when patients have more number of symptoms.

CONCLUSION

The results of this study showed that COVID-19 in children is noted to be more common among males and children one to three years old. Majority of the subjects belong to the mild classification. The most common manifestations were fever and cough. There is no significant difference in the mean Ct values of the different groups of presenting manifestations.

A decreasing trend in the mean Ct values is observed on the first three days of illness and then the values start to rise on the fourth day.

There is an inverse correlation between Ct value and the number of presenting manifestations. The higher number of presenting manifestations is associated with lower Ct values.

RECOMMENDATIONS

For proper interpretation of Ct values, this study emphasizes that it must always be correlated with the patient's exposure history and symptoms. Isolation and early testing for SARS-CoV-2 during the first three days of illness is recommended, especially among patients with respiratory symptoms, as their viral shedding may be the highest. Through early identification and isolation, prevention of transmission may be more effective.

To better understand the disease trajectory and transmission dynamics, conduction of larger, multi-site, prospective studies will help strengthen the results. A prospective study which will compare the Ct values of each patient from the first day of illness and onwards will help analyze infectiousness. Moreover, another study which will monitor the development of symptoms, RT-PCR result and Ct value of COVID patients' close contacts during the pre-symptomatic and symptomatic phase may also be done in order to prove the utility of Ct value as an indirect measure of infectiousness and thereby contribute to understanding transmission.

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CONFLICT OF INTEREST

None declared.

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