A DESCRIPTIVE CROSS-SECTIONAL STUDY ON REVERSE TRANSCRIPTASE POLYMERASE CHAIN REACTION (RT-PCR) CYCLE THRESHOLD LEVEL, MORTALITY AND PEDIATRIC ACUTE RESPIRATORY DISTRESS SYNDROME AMONG COVID-19 PATIENTS ADMITTED AT PCMC

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ABSTRACT

Severe acute respiratory syndrome coronavirus 2 (SARS CoV-2) is a novel pathogen that has rapidly caused a devastating pandemic of Coronavirus disease 2019 (COVID-19). The real time reverse transcriptase polymerase chain reaction cycle threshold values are inversely related to viral load and believed to have a role in terms of mortality and severity of the disease however, there is limited data in children.

OBJECTIVES: This study aims to determine the RT-PCR cycle threshold level in relation to mortality and pediatric acute respiratory distress syndrome (pARDS) among COVID-19 patients admitted at Philippine Children's Medical Center.

METHODS: A cross sectional study was done on patients with RT-PCR confirmed covid-19 admitted at Philippine Children's Medical Center from September 2020 to June 2021.

RESULTS: 50 nasopharyngeal swab specimens from children admitted for COVID-19 were analyzed. 12 (24%) had acute respiratory distress syndrome. Among the 12 children who had pARDS, six (50%) expired; in those without pARDS, two (5.26%) expired. There was no difference in cycle threshold values between patients who died and who survived, as well as those with or without pARDS.

CONCLUSIONS AND RECOMMENDATIONS: We have no evidence to demonstrate a difference in Ct values alone between children who died or survived, or those who developed pARDS or those who did not. RT-PCR cycle threshold alone cannot predict mortality and development of pARDS, it can only indicate the presence of infection but not its severity. Cycle

threshold and its significance may further be explored with a bigger population size in children in future studies.

Keyword/s: *severe acute respiratory syndrome coronavirus 2, RT-PCR cycle threshold, mortality, pediatric acute respiratory distress syndrome*

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS CoV-2) is a novel pathogen that has rapidly caused a devastating pandemic of Coronavirus disease 2019 (COVID-19). It exhibits different disease severity among infected patients, ranging from an absence of symptoms to fatal outcomes.

The gold standard in diagnosing coronavirus disease 2019 is via real-time reverse transcriptase polymerase chain reaction (RT-PCR)[1] from nasopharyngeal and oropharyngeal swab. Given that polymerase chain reaction amplifies a target stretch nucleic acid exponentially, samples which begin the reaction with more abundant target material will produce a detectable signal earlier than samples with lower target abundance. The cycle threshold value (Ct) derived from a sample is essentially a measure of the amplification required for the target viral gene to cross a threshold value and is inversely related to the viral load[2].

The utility of RT-PCR Ct in the management of Covid-19 patients remains controversial. Several published studies on RT-PCR with low Ct in adults showed more serious and greater risk of mortality. A study done by Huang et. al, on RT-PCR Ct value and mortality has been demonstrated the correlation of lower Ct values with high mortality risk. SARS-CoV-2 viral load upon admission among hospitalized patients with COVID-19 independently correlates with the risk of intubation and in- hospital mortality according to Magleby et.al. There are several studies correlating SARS-CoV-2 RT-PCR Ct on mortality and ARDS in adults however, data on mortality and development of pediatric acute respiratory distress syndrome in children with Covid-19 are limited. This study was done to determine the association of RT-PCR Ct with development of pARDS and mortality among admitted patients with

COVID-19 at Philippine Children's Medical Center.

OBJECTIVES OF THE STUDY

Specific Objectives:

To determine whether there is a difference of RT-PCR cycle threshold in terms of:

a. in-hospital mortality
b. pediatric acute respiratory distress syndrome

OPERATIONAL DEFINITIONS OF TERMS AND VARIABLES

All- Cause Mortality: the death rate from all causes of death for a population in a given time period.

ARDS: Acute Respiratory Distress Syndrome.

Base on World Health Organization Clinical Management of COVID -19 Interim Guidance. 2020. Acute Respiratory Distress Syndrome defined as any of the following:

a. Onset: within 1 week of a known clinical insult or new or worsening respiratory symptoms.
b. Chest imaging: (radiograph, CT scan, or lung ultrasound): bilateral opacities, not fully explained by volume overload, lobar or lung collapse, or nodules.
c. Origin of pulmonary infiltrates: respiratory failure not fully explained by cardiac failure or fluid overload.
d. Oxygenation impairment in children:
    1. Bilevel (NIV or CPAP) ≥ 5 cmH2O via full face mask: PaO2/FiO2 ≤ 300 mmHg or SpO2/FiO2 ≤ 264
    2. Mild ARDS (invasively ventilated): 4 ≤ OI < 8 or 5 ≤ OSI < 7.5.
    3. Moderate ARDS (invasively ventilated): 8 ≤ OI < 16 or 7.5 ≤ OSI < 12.3.
    4. Severe ARDS (invasively ventilated): OI ≥ 16 or OSI ≥ 12.3.

COVID 19: Corona Virus Disease 2019, a disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Diagnosed by real time polymerase chain reaction (RT-PCR) and at least one of the following mild clinical symptoms (fever, cough, fatigue, anorexia, shortness of breath, myalgias)

Cycle threshold (Ct) value: specific threshold within a certain number of PCR cycle. Cycle threshold can be defined as the thermal cycle number at which the fluorescent signal exceeds that of the background and passes the threshold for positivity. Typical RT-PCR assay will have a maximum of 40 thermal cycles. The lower the Ct value the higher the quantity of viral genetic material in the sample. Ct values obtained in this way are semi-quantitative and are able to distinguish between high and low viral load. PCR cycle reported as follows based on the machine used at PCMC COVID laboratory.

1. FAM channel (ORF1ab)
   Positive: </= 38
   Negative: > 38 or no Ct value
2. ROX channel (E gene)
   Positive: </=37
   Negative: > 37 or no Ct value
3. Cy5 channel (N gene)
   Positive: </= 38
   Negative: >38 or no Ct value
4. HEX or VIC channel (Internal control)
   Positive: </= 38
   Negative: > 38 or no Ct value

The ORF1ab, N gene, E gene are the tested gene targets for the detection of SARS-Cov-2 at PCMC covid laboratory. The Ct value from each fluorescence channel (ORF1ab, E gene, N gene and Internal control) is labelled positive or negative base on the above cut off Ct value. A test is valid if the internal control result is positive, or the internal control is negative but at least one of the three target channels (ORF1ab, N gene, or E gene) is positive. The test results are interpreted based on the Ct value, a patient sample is labelled as SARS-Cov-2 positive if ORF1ab, N gene, E gene and IC are all (+) or ORF1ab (+), N gene /E gene (any) and Internal control (any).

METHODOLOGY

Study Design

This is a cross sectional study of patients with RT-PCR confirmed covid-19 admitted at Philippine Children's Medical Center from September 2020 to June 2021.

Study Participants

Inclusion/Exclusion Criteria

Subjects included all admitted patients at Philippine Children's Medical Center from September 2020 to June 2021

with a positive RT-PCR SARS- CoV-2 using nasopharyngeal and oropharyngeal swab specimen and presented with mild symptoms of the disease upon admission.

All patients with previous COVID-19 were excluded. Samples collected more than 24 hours upon admission or analyzed outside PCMC were excluded from the study.

Sample Size

No sample size calculation was needed because all RT-PCR confirmed Covid patients were included.

Data Collection and Outcomes

The patient information and data including comorbidities, disease presentation on admission and outcome of patients were collected retrospectively from the medical records. A uniform method of data abstraction was applied which included the following A. Demographic data (1) age (2) biological sex. B. Initial Clinical characteristics upon admission (1) duration of illness (2) presented symptoms such as fever, cough, sore throat, headache, diarrhea. (2) co-morbid conditions – cancer, neurologic, immunodeficient, cardiovascular, chronic lung disease, chronic liver disease and chronic kidney disease. C. Arterial blood gas-Pa02/Fi02 D. ARDS-mild, moderate, severe, died or alive. E. Medications given F. Outcome after 28 days-died, alive.

Nasopharyngeal and oropharyngeal samples collected within 24 hours upon admission was used for this study. Nasopharyngeal and oropharyngeal samples were collected by pediatric residents on duty upon admission. All Specimen collected were submitted to PCMC COVID laboratory for processing. Results of the test released after 24 to 48 hours. Nucleic acid extraction was performed using the machine Alsheng (Hangzhou Allsheng Instruments Co.Ltd). For RT-PCR, MA-6000 machine was used performing the Maccura SARS-CoV-2 RT-PCR kit with 3 primer/probe sets target ORF1ab, N and E gene, respectively. The Ct result value was obtained from the 4 fluorescence channel (ORF1ab, N gene, E gene and Internal Control). Ct value result is positive if ORF1ab less than or equal to 38, N gene less than or equal to 38, E gene less than or equal to 37 and lastly, Internal Control less than or equal to 38.

The cycle threshold value was obtained from the amplification of the

ORF1ab, N gene and E gene. Cycle threshold value from each gene was obtained separately. Median RT-PCR cycle threshold from each gene was determined. The obtained median cycle threshold level from those who died of the disease were determined whether there is difference among those who survived. Among those who survived, median value of RT-PCR cycle threshold who developed acute respiratory distress syndrome were also determined whether there is difference from those who did not.

We used the World Health Organization Clinical Management of COVID -19 Interim Guidance 2020 to diagnosed pediatric acute respiratory distress syndrome as defined in the operational definition section. Patient with pARDS met all the criteria enumerated.

Ethical Considerations

The protocol of this study adheres to the ethical considerations and ethical principles set out in relevant guidelines, including the Declaration of Helsinki, WHO guidelines, International Conference on Harmonization-Good Clinical Practice, Data Privacy Act of 2012, and National Ethics Guidelines for Health Research. The authors report no disclosures.

No potential conflicts of interest have been identified. The principal investigators and co-investigators report no disclosures. This study was fully funded by the author. Subject information was kept in a secure office, with access available only to members of the research team. Computerized study information was stored on a secured network with password access. All identifiable information and data were given a code number. Only members of the research team have access to the list. The research records were stored for at least 6 months following completion of the study. Individually identifiable research data was not shared with others outside of the research and analysis team. The investigator and all key personnel have completed the Good Clinical Practice (GCP) training on the responsible conduct of research with human data.

The study was only commenced upon the approval of the Institutional Review Board. The data were collected through a chart review, precluding informed consent. No adverse events were anticipated, because this is a study conducted retrospectively.

No compensation was given to patients who were part of the research. We recognize that our subjects, patients who tested positive RT-PCR SARS-CoV-2, were particularly vulnerable, and we will take extra care in the confidentiality of their identities. Moreover, this is a retrospective study.

Data Processing and Analysis

Descriptive statistics was used to summarize the general and clinical characteristics of the participants. Frequency and proportion were used for categorical variables. Shapiro-Wilk test was used to determine the normality distribution of continuous variables. Continuous quantitative data that met the normality assumption were summarized using mean and standard deviation (SD), while those that do not were described using median and range.

Continuous variables which were normally distributed were compared using the Independent t-test. Otherwise, the non-parametric Mann-Whitney U test was used. For categorical variables, Chi-square test was used to compare the outcomes. If the expected percentages in the cells are less than 5%, Fisher's Exact test was used instead.

All valid data was included in the analysis. Missing values were neither replaced nor estimated. Null hypothesis was rejected at 0.05α-level of significance. STATA 15.0 (StataCorp SE, College Station, TX, USA) was used for data analysis.

RESULTS

We analyzed a total of 50 nasopharyngeal and oropharyngeal swab specimens from children admitted for COVID-19 from September 2020 to June 2021. Of 50 patients, 12 (24%) had acute respiratory distress syndrome, and 8 died (6 with pARDS, 2 without).

The top three symptoms were fever (50%), cough (24%), and difficulty of breathing (16%). One-fifth of the patients had a neurologic co-morbidity, nine with cancer, and six with chronic kidney disease. Most of the patients are non- oxygen requiring (38%), Arterial blood gases, and treatments received are enumerated on table 2.

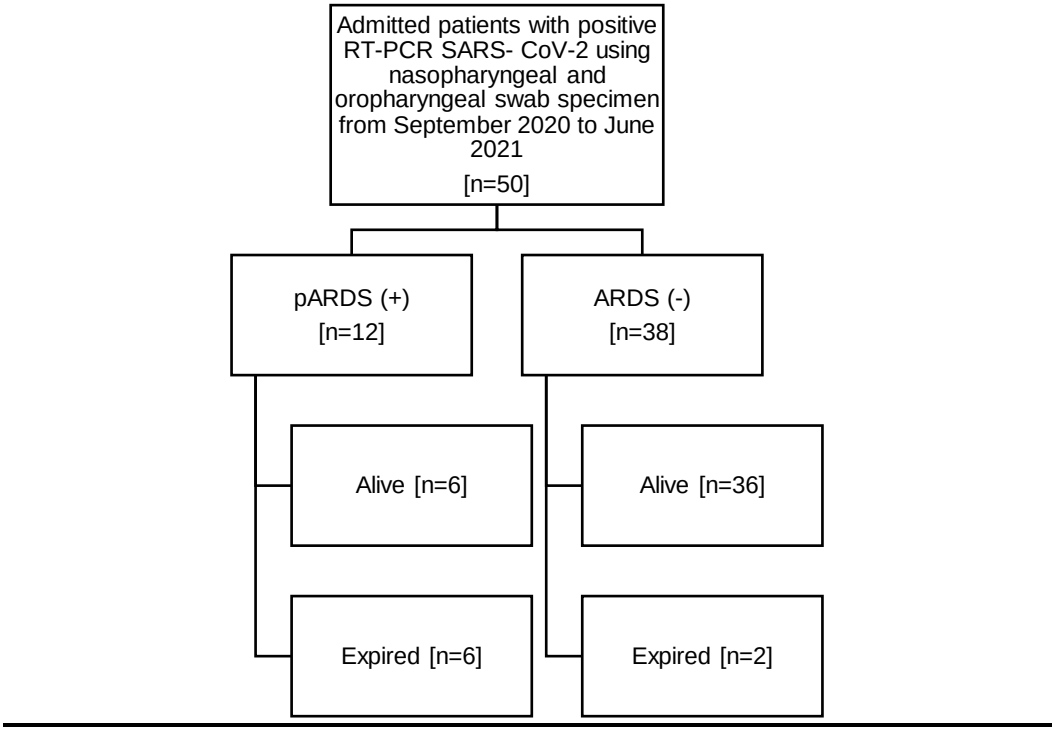

Figure 1. Flowchart of different progression of outcomes

The demographic data is listed in Table 1.

Table 1. Demographic characteristics of patients (n=50)

	Mean (Range); Frequency (%)
Age	
<30 days	1 (2.00)
1 – 11 months	12 (24.00)
1 – 5 years	9 (18.00)
6 – 10 years	16 (32.00)
11 – 15 years	6 (12.00)
16 – 17 years	6 (12.00)
Sex	
Male	34 (68.00)
Female	16 (32.00)

Table 2.Clinical characteristics of patients

	Mean (Range); Frequency (%)
Duration of Illness, days	3 (1-14)
Symptoms	
Fever	25 (50.00)
Cough	12 (24.00)
Sore throat	1 (2.00)
Seizure	7 (14.00)
Chest pain	1 (2.00)
Diarrhea	7 (14.00)
Vomiting	2 (4.00)
Difficulty of breathing	8 (16.00)
Comorbidity	
Cancer	9 (18.00)
CKD	6 (12.00)
Cardiovascular	1 (2.00)
Liver disease	4 (8.00)
Neurologic	10 (20.00)
Hypertension	2 (4.00)
Immunodeficient	1 (2.00)
ABG	
pH	7.36±0.12
PCO2	26.5 (10-80)
PaO2	137.27±65.40
SO2	99 (79-100)
Sodium bicarbonate	16.33±7.14
Base excess	-9.05 (-22.1-3)
O2 requirement	
Room air	19 (38.00)
O2 cannula	6 (12.00)
Face mask	10 (20.00)
NRM	2 (4.00)
BiPAP	3 (6.00)
Nasal CPAP	1 (2.00)
HFNC	4 (8.00)
NIPPV	1 (2.00)
Intubated	4 (8.00)
Medications	
Vitamin D3	43 (86.00)
Zinc	43 (86.00)
Dexamethasone	19 (38.00)
Remdesivir	4 (8.00)
Methylprednisolone	1 (2.00)
Hydrocortisone	1 (2.00)
None	5 (10.00)

RT-PCR Ct for patients with and without pARDS are listed in Table 3. There was no significant difference seen.

RT-PCR Ct for patients who survived and died are listed in Table 4. There was no significant difference seen.

Table 3.SARS-CoV-2 RT-PCR cycle threshold, by pARDS

	pARDS- (n=38)	**pARDS+ (n=12)**	***p***
ORF1ab			
Median (Range)	29.16 (11.70-39.38)	31.07 (12.59-35.77)	.935
Frequency (%)			
≤38	34 (89.47)	10 (83.33)	
>38	4 (10.53)	2 (16.67)	
	30.18 (26.56-38.10)	29.27 (28.11-35.28)	.448
Internal Control			
Median (Range)			
Frequency (%)			
≤38	37 (97.37)	12 (100)	
>38	1 (2.63)	0	
E gene	29.38 (10.13-39.46)		
Median(Range)		31.15 (12.34-39.63)	.778
Frequency (%)			
≤37	35 (92.11)	10 (83.33)	
>37	3 (7.89)	2 (16.67)	
N gene			
Median(Range)	29.52 (8.75-35.67)	31.59 (13.36-36.04)	.601
Frequency (%)			
≤38	37 (97.37)	12 (100)	
>38	1 (2.63)	0	

Table 4. SARS-CoV-2 RT-PCR cycle threshold, by in-hospital mortality

	Alive (n=42)	Expired (n=8)	*p*
ORF1ab			
Median (Range)	29.17 (11.70-39.38)	30.93 (15.23-36.08)	.900
Frequency (%)			
≤38	37 (88.10)	7 (87.50)	
>38	5 (11.90)	1 (12.50)	
			.751
Internal Control			
Median (Range)	30.05 (26.56-38.10)	30.14 (28.23-34.90)	
Frequency (%)			
≤38	41 (97.62)	8 (100)	
>38	1 (2.38)	0	
E gene			
Median (Range)	29.68 (10.13-39.63)	29.90 (13.90-36.43)	.895
Frequency (%)			
≤37	38 (90.48)	7 (87.50)	
>37	4 (9.52)	1 (12.50)	
N gene			
Median (Range)	29.57 (8.75-35.67)	30.20 (15.43-36.04)	.589
Frequency (%)			
≤38	41 (97.62)	8 (100)	
>38	1 (2.38)	0	

DISCUSSION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic has affected the entire world. Clinicians, researcher and scientists are making all efforts to identify ways to diagnose faster, predict outcome and find treatment modalities.

The COVID polymerase chain reaction is the standard test being used for the diagnosis and it gives an additional value known as cycle threshold (Ct), which is the number of PCR cycles required to cross the designated threshold and termed patient as positive for the infection. The Ct value-based estimates of viral load have been used to predict disease progression, infer transmissibility and differentiate active viral replication from prolonged virus shedding.[2]

Several studies on the association of Ct value with mortality and development of respiratory failure has been published. Admission SARS-CoV-2 viral load among hospitalized patients with COVID-19 independently correlates with the risk of intubation and in- hospital mortality.[9-10] Choudhuri et al, SARS-CoV-2 Ct was found to be independent predictor of patient mortality.[11] SARS-Cov-2 Ct value and mortality has been demonstrated the correlation of lower Ct values with high mortality risk.[13]. SARS-CoV-2 viral load, measured by the Ct value of the rRT-PCR in nasopharyngeal swabs on admission, is a viable prognostic marker for the development of respiratory failure.[12]

On the contrary, Cargo et al, studied on Ct values from ORF1ab and S genes and found out that there was no correlation between Ct values for any of these target genes and the oxygen requirements of the patients at the time of sample collection and no difference in the initial nor the nadir Ct values between survivors and non survivors or mild/moderate versus severe/critical illness.[3] Several studies also have shown that nasopharyngeal SARS-CoV-2 Ct values are not associated with COVID- 19 severity and do not support a predictive role for the Ct value in the clinical setting.[4-8] In addition, SARS-CoV-2 Ct values from asymptomatic patients are similar to those in symptomatic patients.[6-8]

In our study, there was no difference in cycle threshold value between patients who died and who survived, and those with and without pARDS. The viral load of SARS-CoV-2 is known to vary during the course of infection.[15] One of the possible reason is the time from onset of symptoms to sampling which is varied between studies and in most of the studies, varied between patients.[16] Furthermore, most literatures correlating Ct value with mortality and pARDS were based on studies with adult subjects. According to Ade et al, age is an important cofactor in SARS-CoV-2 positive patients and may have influence on Ct values in SARS-CoV-2-PCR.[17] Previous studies dealt with older populations and studies on cycle threshold among children are limited and need to investigate more in detail.

CONCLUSION AND RECOMMENDATIONS

Currently , we are not able to demonstrate a difference in Ct values alone

between children who died or survived, or those who developed pARDS or those who did not. Reverse transcriptase polymerase chain reaction cycle threshold alone cannot predict mortality and development of pARDS, it can only indicate the presence of infection but not its severity. Our study was limited with small sample size, cycle threshold and its significance may further be explored with a bigger population size in children in future studies.

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