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Artienda AO, Padua JRO. SARS-CoV-2 RT-PCR cycle threshold value and its association with disease severity and mortality among hospitalized pediatric covid-19 patients. https://doi.org/10.56964/pidspj20232402007

ORIGINAL ARTICLE

SARS-COV-2 RT-PCR CYCLE THRESHOLD VALUE AND ITS ASSOCIATION WITH DISEASE SEVERITY AND MORTALITY AMONG HOSPITALIZED PEDIATRIC COVID-19 PATIENTS

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ABSTRACT

Objective: This study determined the association of SARS-CoV-2 RT-PCR cycle threshold (Ct) value with disease severity and mortality among hospitalized pediatric COVID-19 patients.

Methodology: This is a retrospective cohort study of patients aged 0-18 years with SARS-CoV-2 RT-PCR-confirmed COVID-19 from 1-September-2020 to 31-August-2022. The cohort was divided into those with high (>30), medium (> 20) and low (</= 20) Ct values. Association between Ct values and disease severity was determined using Chi-square test and association between Ct values and mortality was determined using logistic regression.

Results: There were 236 patients included with male predominance. Median age was 7 years. Most belonged to the 0-5 years age group. Most were severe to critical COVID-19 cases. Median day of illness on swab collection was 4 days. Majority presented with symptoms such as fever (54%), cough (22%) and dyspnea (22%). Eighty-four percent had co-morbidities, of which majority were cancer and neurologic diseases. Median Ct value was 30.81. Fifty-four percent had high Ct values. The median age of patients with a high Ct value was significantly lower than other cohorts. The median day of illness of patients with low Ct value was significantly shorter than other cohorts. There was no significant difference across the terciles in terms of presence of co-morbidities. Majority of patients for each cohort had high Ct values. There was no significant association between Ct value and COVID-19 disease severity on admission. Nearly fifty percent had critical disease and the all-cause mortality rate was 21.61%. There was no significant association between Ct value and mortality.

Conclusions: Ct value was not associated with disease severity and all-cause mortality after controlling for confounders. A look into medical interventions, emergence of variants, and other factors that may affect the clinical presentation, disease course, severity and outcome are recommended in future studies.

KEYWORDS: *COVID-19, Cycle Threshold Value, Disease Severity, Mortality, Outcome, Pediatric Patients*

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INTRODUCTION

Coronavirus Disease-2019 (COVID-19) remains a global health concern since the World Health Organization (WHO) declared it a pandemic in March of 2020[1]. As part of the emergency response, countries around the world proposed a series of interim guidelines in line with the WHO's advice regarding disease detection, testing and management.[2] Various efforts to understand this novel disease have driven the medical society to explore on diagnostics for Coronavirus-2 Severe Acute Respiratory Syndrome (SARS-CoV-2), with the hope of improving outcomes among COVID-19 confirmed cases. Disease complexity is manifested through a spectrum of illness severity states—from an asymptomatic or mild infection to severe and critical condition.[3] As the disease continues to evolve, knowledge on pathogenesis and subsequently, developments in diagnostic testing and test interpretation also remain to be partial. It is being explored if the ability to predict disease severity and outcome through these diagnostics would significantly benefit treatment and management decisions.

The Reverse Transcription Polymerase Chain Reaction for SARS-CoV-2 (SARS-CoV-2 RT-PCR) is the gold standard and is the most reliable test for the diagnosis of COVID-19.[4,5] It detects the viral ribonucleic acid semi-quantitatively by providing an indirect measure of the viral load found in the sample.[6] The kits for RT-PCR are designed to recognize a target gene.[6] Once a target gene has been detected, a cycle threshold value (CT value) is recorded which reflects the number of amplification cycles necessary for the recognition of the target gene.[7] An inverse relationship between the Ct value and viral load is observed—the lower the amplification necessary for the machine to detect the virus, the higher the viral load, and vice versa[7]. A low Ct value indicates a high concentration of SARS-CoV-2 genetic material or viral load. Conversely, a high Ct value indicates a low concentration of viral genetic material or viral load. It is uncertain, however, whether this semi-quantitative capability of the SARS-CoV-2 RT-PCR can be maximized.

Several studies have been done to investigate the significance of the Ct value and its association with disease severity and outcome among COVID-19 patients. However, to date, there are limited local studies on Ct value and its association with disease severity and outcome among pediatric patients with COVID-19 and morbidity & mortality from SARS-CoV-2 infection remain to be a concern especially among those with co-morbidities.

This study aimed to determine the association of SARS-CoV-2 RT-PCR Ct value with disease severity and mortality among pediatric COVID-19 patients admitted at the Philippine Children's Medical Center (PCMC). The demographic profile and clinical characteristics of the study population, their Ct values, the association between the Ct value and COVID-19 disease severity, and the association between the Ct value and all-cause mortality during admission were also studied.

MATERIALS AND METHODOLOGY

This is a retrospective cohort study of pediatric patients aged 0 to 18 years with confirmed COVID-19 admitted at the Philippine Children's Medical Center from September 1, 2020 to August 31, 2022. The study period coincided with the start of operations of the COVID-19 Testing Laboratory of the Pathology Division of PCMC.

Patients included in the study were those with positive SARS-CoV-2 RT-PCR result done by the COVID-19 Testing Laboratory as a requirement for admission, regardless of symptomatology. Three FDA-approved SARS-CoV-2 RT-PCR kits were available in the laboratory during the study period, namely the Maccura SARS-CoV-2 Fluorescent PCR, iPonatic 2019-nCoV Kit by Sansure Biotech and Sansure Biotech Novel Coronavirus Nucleic Acid Diagnostic Kit.

These kits used the same principle of a real-time reverse-transcription PCR system, where specific primers and fluorescent probes are used to target the ORF1ab, E and N genes for the Maccura kit; and ORF1ab, N gene, and internal standard gene

fragments of SARS-CoV-2 for the two Sansure Biotech kits. All kits follow the same set cycle parameters of reverse transcription, pre-denaturation, annealing, extension, fluorescence collection and instrument cooling. The thermal cycles are defined as the cycle threshold when the fluorescent signal exceeds the background fluorescence, which is a semi-quantitative measure of viral genetic material in samples. A standard RT-PCR assay runs a maximum of 40 thermal cycles. The interpretation of Ct value of the target gene (ORF1ab) has a cut-off of less than or equal to 38 for the Macurra kit, and less than or equal to 40 for the Sansure kits. Those over this set cut-off values are released as SARS-CoV-2 RNA not detected and interpreted as negative, while those within the cut-off values are released as SARS-CoV-2 RNA detected and interpreted as positive result.

The specimens submitted were nasopharyngeal swab (NPS) and oropharyngeal swab (OPS), NPS alone or OPS alone. Collection of specimen from both NPS and OPS is the standard technique. However, for patients prone to bleeding due to underlying conditions, only OPS was sent. For intubated patients or for those with contraptions that do not permit access to the oral cavity, only NPS was sent. Specimens were immediately submitted to the COVID-19 Testing Laboratory and further evaluated by the laboratory analysts to ensure integrity and adequacy prior to testing. Specimen collection procedures complied with those set by the PCR kit manufacturers.

The definitions provided by the Interim Guidelines on the Screening, Assessment and Clinical Management of Pediatric Patients with Suspected or Confirmed Coronavirus Disease-2019 of the Philippine Pediatric Society and Pediatric Infectious Disease Society of the Philippines were used to categorize cases. Those with mild disease were symptomatic patients meeting the case definition for COVID-19 without evidence of viral pneumonia or hypoxia[3]. Moderate disease included those with clinical signs of non-severe pneumonia such as absence of fast breathing, difficulty of breathing, chest indrawing or desaturation less than 90% on room air[3]. Severe disease included those with clinical signs of pneumonia such as cough or difficulty of breathing plus at least one of the following: central cyanosis or oxygen saturation less than 90%, poor intake, lethargy and unconsciousness, or convulsions. Lastly, critical disease included patients who presented with acute respiratory distress syndrome, sepsis, septic shock, acute thrombosis or multi-system inflammatory syndrome in children.[3]

Patient outcomes were classified as survival (discharged improved) or mortality for those who succumbed to death regardless of cause while admitted.

Due to the finite number of eligible patients in PCMC, the researcher employed a total enumeration technique, a type of purposive sampling design wherein all eligible patients were enrolled in the study. PASS 15 software was used to calculate the sample size requirement to achieve 80% statistical power. Parameters were based on the published study by Klinger, *et al.*[8] Specifying an odds ratio of 2.93, probability of mortality among low Ct value patients equal to 22%, and alpha set at 0.05, a minimum of 66 patients were required to achieve 80% statistical power.

There was no randomization and intervention employed. The study was approved by the ethics committee of PCMC prior to its implementation. Data gathering was done by chart review and there was no direct encounter with patients or guardians hence a waiver of consent was applied.

There were 360 patients who tested positive from September 2020 to August 2022 based on the records of the Infection Prevention and Control Committee of PCMC. This list was submitted to the COVID-19 Testing Laboratory for retrieval of Ct values. Further information was gathered for the 360 patients from the database of the Section of Pediatric Infectious Diseases and supplemented by chart review. Eleven cases were still admitted at the end of the data collection period, hence these were excluded in the study. From the 349 remaining subjects, only 236 had retrievable records with complete data, including age, gender, symptoms (if

any), day of illness on the day of swab, disease severity, co-morbidities (if any) and outcome. From 236 subjects, 170 were tested using the Maccura kit, 59 using the iPonatic kit by Sansure Biotech and 7 using the 2019-nCoV Nucleic Acid Diagnostic kit by Sansure Biotech.

The cohort was divided into terciles based on Ct values for the SARS-CoV-2 -specific target (ORF1ab). High Ct value included those with Ct value more than 30, medium included those with more than 20 and low included those with less than or equal to 20.

Data were recorded by the researcher in a data collection form and encoded in Microsoft Excel. Stata MP version 17 software was used for data processing and analysis. Continuous data were presented as median/interquartile range due to the non-normal data distribution. Categorical data were presented as frequencies and percentages. Kruskal Wallis test was used to compare continuous variables by Ct value, while Chi-Square test and Fisher's Exact test were used for categorical variables. Comparison of characteristics by mortality status was performed using Mann-Whitney U test for continuous variables, and Chi-Square and Fisher's Exact test were used for categorical variables.

The association between Ct value and disease severity was determined using Chi-square test. Logistic regression analysis was performed to determine the association between Ct value and mortality, and in case of sparse data, Firth's bias correction was applied. Confounder selection utilized a cut-off of $p<0.20$ and change-in-estimate criterion of 10%,[9] and p values ≤0.05 were considered statistically significant.

RESULTS

A total of 236 pediatric patients were included in the study. Table 1 shows the baseline demographic and clinical characteristics of patients. The median age was 7 years old (range: 1 day to 18 years old), and most patients belonged to the 0 to 5 years age group, with an interquartile range (IQR) of 1 to 3 years. Majority were males. More than half were severe to critical COVID-19 cases. The median day of illness at the time of swab collection was 4 days (range: 0 to 56 days; IQR: 2 to 7 days).

Table 1. Baseline demographic and clinical characteristics of patients (n=236)

	n (%)
Age (in years), median	7 [IQR: 1-3]
0-5 years old	106 (45)
6-10 years old	54 (23)
11-15 years old	47 (20)
16-18 years old	29 (12)
Gender	
Male	145 (61)
Female	91 (39)
COVID-19 severity on diagnosis	
Asymptomatic	22 (9)
Mild	69 (29)
Moderate	24 (10)
Severe	60 (25)
Critical	61 (26)
Day of illness at the time of swab	
Days, median	4 [IQR: 2-7]
Symptoms	
With	214 (91)
Without	22 (9)
Co-morbidities	
With	199 (84)
Without	37 (16)

Majority of patients had symptoms and more than half had fever followed by cough (22%), dyspnea (22%), vomiting (18%), and seizures (17%). The most common organ systems involved were the respiratory (55%), neurologic (25%), gastrointestinal (17%) and hematologic (16%) systems. Thirty-eight percent of patients had multi-organ involvement.

Table 2. List of specific symptoms of pediatric COVID-19 patients (n=236)

Symptoms	n (%)
With	214 (91)
Without	22 (9)
Specific symptoms	
Fever	128 (54)
Cough	54 (22)
Dyspnea	53 (22)
Vomiting	43 (18)
Seizure	41 (17)
Bleeding	27 (11)
Anorexia	24 (10)

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Table 2 continued. List of specific symptoms of pediatric COVID-19 patients (n=236)

Specific symptoms	
Abdominal pain	21 (9)
Colds	19 (8)
Headache	15 (6)
Diarrhea	14 (6)
Body pains	11 (5)
Edema	11 (5)
Easy fatigability	11 (5)
Pallor	8 (3)
Poor activity/ decreasing sensorium	8 (3)
Rashes	7 (3)
Irritability	6 (3)
Weight loss	3 (1)
Cyanosis	3 (1)
Dysuria	3 (1)
Oliguria	2 (1)
Sore throat	2 (1)
Apnea	1 (1)
Oral sores	1 (1)

Eighty-four percent of patients had co-morbidities and most common were cancer (24%) and neurologic (16%) diseases as listed in Table 3.

Majority of patients (72%) were tested for RT-PCR using the Maccura kit, the first utilized in the hospital, followed by the iPonatic (25%), and Sansure kit (3%).

Table 3. List of specific co-morbidities of pediatric COVID-19 patients (n=236)

Co-morbidities	n (%)
With	199 (84)
Without	37 (16)
Specific co-morbidities	
Cancer	57 (24)
Neurologic	37 (16)
Co-infection	24 (10)
Gastrointestinal	21 (9)
Renal	17 (7)
Congenital anomaly	16 (7)
Hematologic	13 (6)
Cardiac	8 (3)
Respiratory	7 (3)
Others	11 (5)

The median Ct value was 30.81 [IQR: 20.48-35.81, range of 10.13-44.29]. Fifty- four percent had high Ct values, 23% had medium Ct values and the remaining 23% had low Ct values. Table 4 compares the patient characteristics by Ct value.

The median age significantly differ by Ct values. The median age of patients with high Ct value was significantly lower than those with low (p=0.0229) and medium (p=0.0084) Ct values. There was no significant difference between the low and medium Ct value groups (p=0.3617). The age groups also showed significant differences by Ct value. A higher proportion of patients with high Ct values belong to the 0 to 5 year age group compared to those with low and medium Ct values.

The median day of illness at the time of swab was significantly different by Ct values. The median day of illness of patients with low Ct values was significantly shorter compared to those with medium Ct (p=0.0007) and high Ct values (p=0.0118). There was no significant difference in the median day of illness between those with medium and high Ct values (p=0.0608).

Table 4. Demographic profile, clinical characteristics and SARS-CoV-2 RT-PCR Ct Values of pediatric COVID-19 patients (n=236)

	Ct value [n (%)]			
	Low (n=55)	**Medium (n=53)**	**High (n=128)**	***p* value**
Age (years), median	9 [IQR:1-14]	9 [IQR: 4-14]	5 [IQR: 0.79-10]	0.0232*[a]
0 to 5	22 (40)	18 (34)	66 (52)	0.042*[b]
6 to 10	9 (16)	14 (26)	31 (24)	
11 to 15	14 (26)	16 (30)	17 (13)	
16 to 18	10 (18)	5 (10)	14 (11)	
Gender				
Male	30 (55)	33 (62)	82 (64)	0.475[b]
Female	25 (45)	20 (38)	46 (36)	
Day of illness at the time of swab				
Days, median	3 [IQR: 1-4]	5 [IQR: 3-9]	4 [IQR: 2-7]	0.0052*[a]
Symptoms				
With	52 (95)	52 (98)	110 (86)	0.020*[b]
Without	3 (5)	1 (2)	18 (14)	
Co-morbidities	0	0	3 (2)	0.422[c]
With	47 (85)	43 (81)	109 (85)	0.768[b]
Without	8 (15)	10 (19)	19 (15)	

Table 4. Demographic profile, clinical characteristics and SARS-CoV-2 RT-PCR Ct Values of pediatric COVID-19 patients (n=236)

Organ systems involved during the clinical course				
With	46 (84)	48 (91)	107 (84)	0.455[b]
Without	9 (16)	5 (9)	21 (16)	

[a]Kruskal Wallis test was used. Significant results further analyzed using Dunn's test; [b]Chi square test was used; [c]Fisher's exact test was used

The presence of symptoms significantly differ by Ct value as summarized in Table 5. A higher proportion of patients with symptoms had low to medium Ct values compared to other groups. Across all symptoms, only sore throat was significantly different by Ct value. A higher proportion of cases with medium Ct values experienced sore throat than other groups.

Table 5. Specific symptoms and Ct values (n=236)

Specific symptoms, %yes	Ct value [n (%)] Low (n=55)	Medium (n=53)	High (n=128)	*p* value
Headache	4 (7)	0	11 (9)	0.067[c]
Irritability	2 (4)	1 (2)	3 (2)	0.862[c]
Body pains	1 (2)	2 (4)	8 (6)	0.462[c]
Poor activity/ decreasing sensorium	1 (2)	1 (2)	6 (5)	0.704[c]
Easy fatigability	1 (2)	1 (2)	9 (7)	0.278[c]
Anorexia	1 (2)	7 (13)	16 (13)	0.064[b]
Vomiting	9 (16)	12 (23)	22 (17)	0.633[b]
Weight loss	0	2 (4)	1 (1)	0.176[c]
Colds	5 (9)	4 (8)	10 (8)	0.949[c]
Dyspnea	14 (25)	14 (26)	25 (20)	0.499[b]
Apnea	1 (2)	0	0	0.458[c]
Cyanosis	1 (2)	1 (2)	1 (1)	0.594[c]
Diarrhea	6 (11)	2 (4)	6 (5)	0.223[c]
Sore throat	0	2 (4)	0	0.050*[c]
Oral sores	0	1 (2)	0	0.225[c]
Cough	14 (25)	17 (32)	23 (18)	0.106[b]
Bleeding	9 (16)	7 (13)	11 (9)	0.286[b]
Fever	36 (65)	31 (58)	61 (48)	0.067[b]
Seizure	10 (18)	9 (17)	22 (17)	0.983[b]
Abdominal pain	2 (4)	7 (13)	12 (9)	0.187[c]
Pallor	3 (5)	3 (6)	2 (2)	0.141[c]
Rashes	1 (2)	1 (2)	5 (4)	0.777[c]
Edema	1 (2)	3 (6)	7 (5)	0.602[c]
Oliguria	0	0	2 (2)	1.000[c]
Dysuria	0	0	3 (2)	0.422[c]

There was no significant difference across the terciles in terms of presence of co-morbidities. However, when specific co-morbidities were analyzed, significant differences were observed for co-infections as seen in Table 6. A higher proportion of cases with co-infection had high Ct values.

Table 6. Specific co-morbidities and Ct values of pediatric COVID-19 patients (n=236)

Specific co-morbidities, %yes	Ct value [n (%)] Low (n=55)	Medium (n=53)	High (n=128)	*p* value
Neurologic	12 (22)	4 (8)	21 (16)	0.118[b]
Cancer	13 (24)	15 (28)	29 (23)	0.708[b]
Hematologic	5 (9)	3 (6)	5 (4)	0.326[c]
Respiratory	2 (4)	2 (4)	3 (2)	0.673[c]
Co-infection	1 (2)	4 (8)	19 (15)	0.022*[b]
Congenital anomaly	4 (7)	1 (2)	11 (9)	0.270[c]
Renal	3 (5)	4 (8)	10 (8)	0.891[c]
Gastrointestinal	5 (9)	5 (9)	11 (9)	1.000[b]
Cardiac	3 (5)	2 (4)	3 (2)	0.494[c]
Others	2 (4)	4 (8)	5 (4)	0.553[c]

There was no significant difference across the three groups in terms of sex and specific organs involved during the course of COVID-19 illness.

As to disease severity, more than half of patients for each COVID-19 category have high Ct values (Table 7). There was no significant association between Ct value and COVID-19 severity on admission.

Table 7. Association between Ct value and COVID-19 severity (n=236)

COVID-19 severity on admission	Ct value [n (%)] Low (n=55)	Medium (n=53)	High (n=128)	*p* value
Asymptomatic/ Mild	24 (26)	17 (19)	50 (55)	
Moderate/ Severe	20 (24)	21 (25)	43 (51)	0.679[a]
Critical	11 (18)	15 (25)	35 (57)	

[a]Chi-square test was used

As to mortality, 51 patients died, with an all-cause mortality rate of 21.61% (95% CI: 16.80-

27.35%). Incidence of mortality by COVID-19 severity is as follows: asymptomatic/mild: 9.89% (95% CI: 5.18-18.07%), moderate/severe: 14.29% (95% CI: 8.23-23.66%), critical: 49.18% (95% CI: 36.70-61.76%).

Table 8 presents the association between Ct value and mortality. There was no significant association between Ct value and mortality even after controlling for the confounding effect of age. Other potential confounders have been screened and eliminated by simple logistic regression or univariable analysis. A crude odds ratio is generated and *p* value <0.20 is considered as a potential confounder and is entered into the multiple logistic regression model together with Ct value to generate an adjusted OR. Change-in-estimate (CIE) criterion is used to check if these are true confounders. The crude OR of the Ct value is compared to the adjusted OR. After analyses of possible confounders, only age was considered.

Table 8. Association between Ct value and mortality (n=236)

Ct value	CRUDE OR (95% CI)[a]	*p* value	ADJUSTED OR (95% CI)[b]	*p* value
High	*Ref*	*Ref*	*Ref*	*Ref*
Medium	1.15 (0.53-2.49)	0.726	1.30 (0.58-2.90)	0.528
Low	1.21 (0.57-2.59)	0.615	1.17 (0.54-2.56)	0.687

Ref: Reference category; [a] Simple logistic regression analysis; [d]Multiple logistic regression analysis controlled for the confounding effect of age group

Table 9 presents the association between Ct value and mortality in patients by COVID-19 disease severity. Even after controlling for the confounding effect of age, there was no significant association observed between Ct value and mortality among asymptomatic/mild and moderate/severe cases. There was also no significant association observed between Ct value and mortality among critical COVID-19 cases. Furthermore, no significant confounder was recorded, thus, multiple logistic regression analysis was not performed.

Table 9. Association between Ct value and mortality by COVID-19 severity (n=91)

Ct value	CRUDE OR (95% CI)[a]	*p* value	ADJUSTED OR (95% CI)[b]	*p* value
Asymptomatic/Mild				
High	*Ref*	*Ref*	*Ref*	*Ref*
Medium	1.53 (0.25-9.23)	0.641	2.46 (0.43-14.12)	0.312
Low	1.64 (0.34-8.00)	0.539	1.91 (0.36-10.06)	0.443
Moderate/Severe				
High	*Ref*	*Ref*	*Ref*	*Ref*
Medium	1.09 (0.27-4.49)	0.903	1.28 (0.30-5.26)	0.762
Low	1.15 (0.28-4.76)	0.843	1.03 (0.25-4.33)	0.964
Critical				
High	*Ref*	*Ref*	-	-
Medium	1.04 (0.32-3.40)	0.945	-	-
Low	1.97 (0.52-7.52)	0.321	-	-

Ref: Reference category; [a] Simple logistic regression analysis with Firth's bias correction; [b]Multiple logistic regression analysis with Firth's bias correction

DISCUSSION

Our study showed that pediatric COVID-19 patients admitted in PCMC, were comprised mostly of children 0 to 5 years old, with a median age of 7, and predominantly males. This profile is similar to a retrospective study which looked into epidemiological characteristics of pediatric COVID-19 patients in China.[10] In terms of disease severity, however, they have observed more asymptomatic, mild or moderate cases,[10] while our study observed more severe to critical cases. This may be attributed to a skewed population of admitted cases with co-morbidities (84%) as PCMC is a tertiary multi-

specialty referral hospital. This trend in demographics and clinical characteristics was reflected in the study of Gonzales-Ritona in the same institution.[11] Nevertheless, a larger number of studies still support the finding that the pediatric population generally present with milder disease course compared to their adult counterparts.[10,12-13]

As to clinical presentation, fever and cough were the most commonly seen symptoms in our study. This is comparable to studies done previously in children abroad. [12-15] This also conforms with the latest case bulletin released by the Surveillance and Analysis of COVID-19 in Children Nationwide (SALVACION) of the Pediatric Infectious Disease Society of the Philippines (PIDSP). From this registry, the most common symptoms identified in Filipino children were fever at 57.6% followed by cough at 45.2%.[16] Additionally, other symptoms found in our study were dyspnea, vomiting and seizures. These symptoms were also reflected in the SALVACION registry and other pediatric databases.[15-16]

The organ systems most often involved during the course of COVID-19 in this study were the respiratory, neurologic, and gastrointestinal systems with more than a quarter of patients having multi-organ involvement. It is known that in those who acquired COVID-19, the respiratory system is most commonly affected.[17-18] The SARS-CoV-2 binds to angiotensin converting enzyme-2 receptors which are most abundant in the lungs.[17] However, these receptors are also present elsewhere in the body including the heart, vascular endothelial cells, brain, kidneys, intestines, liver, pharynx, and other tissues, thus, directly injuring these other organs as well.[15,17] This explains the multi-organ involvement in COVID-19. Neurological presentations may be due to accompanying fever or the direct viral invasion of the central nervous system since SARS-CoV-2 binds to the ACE-2 receptors found in the cerebral vascular endothelium.[15,17] Coagulopathy leading to infarction is also a plausible mechanism.[15] As for gastrointestinal manifestations, besides the greater expression of ACE-2 receptors in the liver and intestinal tract, it was suggested that the fecal-oral route in disease transmission accounted for the higher incidence of gastrointestinal symptoms in some studies.[15,19.]

When the Ct value is taken into account, we found that age significantly differed across the Ct value terciles, and that younger patients have higher Ct values which indicate lower viral loads compared to children five years and older. Published studies about this association have conflicting findings. In support of our study, one research with a small study population concluded that older patients tend to have higher viral loads.[20] Several postulates on host factors were explored in relation to this including the capacity to form antibodies and the maturity of the immune system. Children often contract viral respiratory infections hence produce more antibodies against viruses compared to adults.[10] Because of this, the immune systems of children are thought to respond to viral infections better.[10] Moreover, since children's immune systems are still underdeveloped, they may react to diseases differently from adults.[10] Due to this immaturity , the binding ability of their ACE-2 receptors may be less efficient compared to adults, therefore, children tend to have less sensitivity to SARS-CoV-2.[18] Also, since there is inefficient binding to receptors , the virus becomes incapable of replication,[18,21] hence, younger children have lower viral load. However, contrary to our study, one small-scale research found that children younger than 5 years of age have low Ct values, hence have higher viral loads.[22] Several other large-scale studies in children and adults found no association between age and viral load.[23-25]

Another significant finding in our study was that the Ct value for patients whose specimens were collected on the third day of illness, were lower than those collected later in the course of the disease. This is congruent with the findings of Zou, *et al.,* wherein higher viral loads were detected soon after symptom onset.[26] This information coincides with the phase of the disease and may aid clinicians in ascertaining whether the patient is in the first phase of illness (about the first week) wherein viremia is expected, or if the patient is in the second phase of illness when

viremia starts to declines and inflammation occurs.[27] This is relevant for clinicians in making treatment decisions, *i.e.* whether to give anti-viral medications, anti-interleukin 6 or steroids when a patient is expected to enter a cytokine storm or during a macrophage activation syndrome.[24]

Pediatric patients in our study who had symptoms had lower Ct values compared to asymptomatic patients. The finding of Roversi, *et al.* strongly suggested that the lack of overt symptomatology in children may be associated with a higher Ct value.[28] However, a number of studies still found that Ct values are comparable between symptomatic and asymptomatic patients, supporting the potential transmission of COVID-19 from asymptomatic children.[29-31]

As for co-morbidities, our study found that patients with co-infections had high Ct values hence a lower viral load. Co-infections play an important role in reducing or augmenting disease severity.[32] In our study, majority of co-infections were viral in etiology. The lower viral load represented by higher Ct values may be explained by viral interference.[33] This phenomenon occurs when one virus influences the replication of the other.[33] The mechanism is also mediated by various factors such as interferons, defective interfering particles, production of trans-acting proteases, cellular factors, and non-specific double-stranded RNA.[34] The host's immune system also affects the outcome of viral co-infections.[33] After exposures to antigens, naive T cells convert into activated effector T cells and later on into long-term memory T cells.[34] Memory responses created to act upon one infection may influence the performance of the immune response to a subsequent secondary infection which is known as heterologous immunity.[34-35] Several immune cells are involved in heterologous immunity and these may result in either a protective or immunopathological response.[35]

Few studies showed an association between Ct values and disease severity.[36-39] In a study by Maltezou, *et al.*, high viral load was inversely correlated with COVID-19 severity across the cohort and in the subgroup of hospitalized patients, even after adjusting for several patient characteristics.[36] Their findings could be useful in the identification of those patients at risk for severe illness or mortality.[36] However, our study did not show significant association between SARS-CoV-2 RT-PCR Ct value and COVID-19 disease severity as well as mortality. This is similar to some larger studies done among pediatric patients.[40-44] A possible explanation for the conflicting results with the association of Ct value and disease severity is the variability in definition of disease severity in different studies[42] In our study, the disease severity classification was based on international and local interim guidelines that evolved throughout the study period encompassing various surges of different variants of SARS-CoV-2.

As for the lack of association of Ct value with mortality, interventions, such as antiviral drugs, immunomodulators, corticosteroids and even vaccination could have had an impact on disease outcome.[42,45] These interventions have been used in some patients during the latter part of the pandemic and those who acquired the disease earlier may not have received them. In our study, the different interventions were not accounted for, hence further studies are recommended to explore their effect on Ct values and disease outcomes.

There were various limitations in our study. This is a retrospective research hence the information gathered depended on what was available on patients' records. As PCMC is a tertiary referral hospital catering mostly to pediatric patients with co-morbidities and those needing specialty care, the results of this study cannot be generalized to other institutions. Moreover, while the study period covered the emergence of different SARS-CoV-2 variants and subvariants, the potential impact of these viral mutations on disease severity and outcome were not included in the analysis. Additionally, among patients with co-morbidities, the causal relationship between COVID-19 and mortality was not explored and established. Lastly, since information about COVID-19 is still evolving, the interventions offered are changing as well. It may be

useful to account for various interventions done on patients that may have affected their clinical presentation, altered the clinical course or affected the severity and outcome of the disease.

CONCLUSION

This study showed no significant association between SARS-CoV-2 RT PCR cycle threshold value with disease severity and in-hospital all-cause mortality among hospitalized pediatric COVID-19 patients. However, younger pediatric patients particularly those five years and below have higher Ct values which indicate lower viral loads. The Ct values of pediatric patients are significantly lower when specimens were collected in the first 3 to 5 days of illness compared to 5 days beyond symptom onset. Pediatric patients with symptoms also had lower Ct values compared to other groups. Lastly, we found that patients with co-infections had higher Ct values.

CONFLICT OF INTEREST

None declared.

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REFERENCES

1. World Health Organization [Internet]. WHO Director-General's opening remarks at the media briefing on COVID-19 - 11 March 2020 (2020 March 11). Retrieved May 5, 2021. Available from: https://www.who.int/director-general/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020
2. McIntosh K. Coronavirus disease 2019 (COVID-19): Epidemiology, virology, and prevention. In A. Bloom (Ed.), UpToDate. (2021 June 9). Retrieved July 8, 2021. Available from: https://www.uptodate.com/contents/covid-19-epidemiology-virology-and-prevention#topicContent
3. Philippine Pediatric Society and Pediatric Infectious Disease Society of the Philippines. (2021 February 6). Interim Guidelines on the Screening, Assessment and Clinical Management of Pediatric Patients with Suspected or Confirmed Coronavirus Disease 19. 2022 January 08. Version 6. 13-16
4. Padua Jr., R. Diagnostic Testing Strategies to Manage COVID-19 Pandemic: Proposed by the Philippine Society of Pathologists, Inc. (2020). PJP, 5(1), 5-8. Available from: https://doi.org/10.21141/PJP.2020.08
5. Sethuraman N, et al. Interpreting Diagnostic Tests for SARS-CoV-2. JAMA [Internet]. 2020 (2020 May 6).;323(22):2249–2251. Available from: doi:10.1001/jama.2020.8259
6. Stites EC, Wilen CB. The Interpretation of SARS-CoV-2 Diagnostic Tests, Med. [Internet] (2020). Available from: https://www.sciencedirect.com/science/article/pii/S2666634020300167
7. Bustin SA, Mueller R. Real-time Reverse Transcription PCR (qRT-PCR) and Its Potential Use in Clinical Diagnosis. [Internet]. (2005). Available from: https://pubmed.ncbi.nlm.nih.gov/16171460/
8. Faico-Filho KS, et al. Is Higher Viral Load in SARS-CoV-2 Associated with Death? The American Society of Tropical Medicine and Hygiene. [Internet]. (2020).
9. Greenland, S., & Pearce, N. Statistical foundations for model-based adjustments. Annual review of public health, [Internet]. (2015). 36, 89–108. Available from https://doi.org/10.1146/annurev-publhealth-031914-122559
10. Yuanyuan Dong, MD, et. Al. Epidemiology of COVID-19 Among Children in China. [Internet]. (2020). Available from: http://publications.aap.org/pediatrics/article-pdf/145/6/e20200702/1080196/peds_20200702.pdf
11. Gonzales-Ritona J. Clinical Presentation and Outcome of Pediatric COVID-19 Patients admitted in Philippine Children's Medical Center (PCMC): The First 100 Cases. [Internet]. (April 2021). Available from: http://www.pcmc.gov.ph/images/RF2021/Poster_Fellows/GONZALES-RITONAJENNEELYNA.pdf
12. Gotzinger F, Begoña S, et al. COVID-19 in children and adolescents in Europe: a multinational, multicentre cohort study. The Lancet, Child and Adolescent Health. [Internet]. (2020 September). Available from: https://doi.org/10.1016/S2352-4642(20)30177-2
13. Ranabothu, S., Onteddu, S., Nalleballe, K., et al. Spectrum of COVID-19 in children. Acta paediatrica [Internet]. (2020). (Oslo, Norway : 1992), 109(9), 1899–1900. Available from: https://doi.org/10.1111/apa.15412
14. Zimmermann P, Curtis N. COVID-19 in Children, Pregnancy and Neonates: A Review of Epidemiologic and Clinical Features. The Pediatric Infectious Disease Journal

[Internet]. (2020 June). - Volume 39 - Issue 6 - p 469-477. Available from: DOI: 10.1097/INF.0000000000002700

15. Sayed M.A., Abdelhakeem M. Typical and Atypical Clinical Presentation of COVID-19 infection in children in the top of pandemic in EL-Minia governorate (two center experience). Mediterr J Hematol Infect Dis. [Internet]. (2022). 14(1): e2022002. Available from: DOI: http://dx.doi.org/10.4084/MJHID.2022.002
16. Chiong-Rivera A, Pantig F, Maramba-Lazarte C, et al. Surveillance and Analysis of COVD-19 in Children Nationwide (SALVACION), Case Bulletin #5. [Internet]. (2002). Available from: http://www.pidsphil.org/home/wp-content/uploads/2022/05/Salvacion20casebulletin205.pdf
17. Jain U. Effect of COVID-19 on the Organs. Cureus, [Internet]. (2020). 12(8), e9540. Available from: https://doi.org/10.7759/cureus.9540
18. Fang F, Lu X. Facing the pandemic of 2019 novel coronavirus infections: the pediatric perspectives [Internet]. (2020 February 2]. 58(0):E001
19. Zimmermann P, Curtis N. Coronavirus infections in children including COVID-19: an overview of the epidemiology, clinical features, diagnosis, treatment and prevention options in children. Pediatr Infect Dis J. [Internet]. 2020. 39:355–368. Available from: doi: 10.1097/INF.0000000000002660.
20. To KK, Tsang OT, Leung WS, et al. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS- CoV-2: an observational cohort study. Lancet Infect Dis. [Internet]. (2020). 20:565–74. Available from: DOI: 10.1016/S1473-3099(20)30196-1
21. Pierce C.A., Sy S., Galen B., et al. Natural mucosal barriers and COVID-19 in children. JCI Insight. 2021;6:eabd5487.
22. Heald-Sargent T, Muller WJ, Zheng X, et al. Age-related differences in nasopharyngeal Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) levels in patients with mild to moderate Coronavirus Disease 2019 (COVID-19). JAMA Pediatr. [Internet]. (2020). 174:902–3. Available from: DOI: 10.1001/jamapediatrics.2020.3651
23. Mahallawi WH, Alsamiri AD, Dabbour AF, et al. Association of Viral Load in SARS-CoV-2 Patients With Age and Gender. Front. Med. [Internet]. (2021). 8:608215. Available from: DOI: 10.3389/fmed.2021.608215
24. Jacot D, Greub G, Jaton K, Opota O. Viral load of SARS-CoV-2 across patients and compared to other respiratory viruses. Microbes Infect. [Internet]. (2020). 22:617–21. Available from: DOI: 10.1016/j.micinf.2020.08.004
25. Baggio S, L'Huillier AG, Yerly S, et al. SARS-CoV-2 viral load in the upper respiratory tract of children and adults with early acute COVID-19. Clin Infect Dis. [Internet]. (2020). 6:ciaa1157. Available from DOI: 10.1101/2020.07.17.20155333
26. Zou, L., Ruan, F., Huang, M., et al. SARS-CoV-2 Viral Load in Upper Respiratory Specimens of Infected Patients. The New England journal of medicine, [Internet]. (2020); 382(12), 1177–1179. Available from: https://doi.org/10.1056/NEJMc2001737
27. Tay M.Z., Poh C.M., Renia L., et al. The trinity of COVID-19: immunity, inflammation and intervention. Nat Rev Immunol. (2020);20(6):363–374.
28. Roversi, M., Coltella, L., Piccioni, L. et al. Relationship between viral load and symptoms in children infected with SARS-CoV-2. Pediatr Res. [Internet]. (2022). Available from: https://doi.org/10.1038/s41390-022-02293-4
29. Qian Wu, Lixia Shi, Haomin Li, et al. Viral RNA Load in Symptomatic and Asymptomatic COVID-19 Omicron Variant-Positive Patients", Canadian Respiratory Journal, [Internet] (2022). vol., Article ID 5460400, 6 pages, 2022. Available from: https://doi.org/10.1155/2022/5460400
30. Ra SH, Lim JS, Kim G, et al. Upper respiratory viral load in asymptomatic individuals and mildly symptomatic patients with SARS-CoV-2 infection. Thorax [Internet]. . (2021 May). 2021;76:61-63.
31. Zuin M, Gentili V, Cervellati C, Rizzo R, Zuliani G. Viral Load Difference between Symptomatic and Asymptomatic COVID-19 Patients: Systematic Review and Meta-Analysis. Infect Dis Rep.[Internet]. (2021 July). 13(3):645-653. Available from: doi: 10.3390/idr13030061. PMID: 34287354; PMCID: PMC8293148.
32. Asner SA, Science ME, Tran D, Smieja M, Merglen A, Mertz D. Clinical disease severity of respiratory viral co-infection versus single viral infection: a systematic review and meta-analysis. PLoS One [Internet]. 2014.9:e99392. Available from: DOI: 10.1371/journal.pone.0099392.
33. Kumar N, Sharma S, Barua S, et al. Virological and immunological outcomes of coinfections. Clin Microbiol Rev. [Internet]. (2018) 31:17. Available from: DOI: 10.1128/CMR.00111-17
34. Salas-Benito JS, De Nova-Ocampo M. Viral interference and persistence in mosquito-borne flaviviruses. J Immunol Res [Internet]. 2015:873404. Available from: DOI: 10.1155/2015/873404.
35. Sharma S, Thomas PG. The two faces of heterologous immunity: protection or immunopathology. J Leukoc Biol [Internet]. (2014). 95:405–416. Available from: DOI: 10.1189/jlb.0713386.
36. Maltezou, H. C., Raftopoulos, V., Vorou, R., et al. Association Between Upper Respiratory Tract Viral Load, Comorbidities, Disease Severity, and Outcome of Patients With SARS-CoV-2 Infection. The Journal of infectious diseases, [Internet]. (2021). 223(7), 1132–1138. Available from: https://doi.org/10.1093/infdis/jiaa804

37. Souverein, D. Stralen, K. Lelyveld, S. et al. Initial Severe Acute Respiratory Syndrome Coronavirus 2 Viral Load Is Associated With Disease Severity: A Retrospective Cohort Study, Open Forum Infectious Diseases, [Internet]. (2022 July). Volume 9, Issue 7. Available from: https://doi.org/10.1093/ofid/ofac223
38. Shah, V., Farah, W., Hill, J. Association Between SARS-CoV-2 Cycle Threshold Values and Clinical Outcomes in Patients With COVID-19: A Systematic Review and Meta-analysis, Open Forum Infectious Diseases, [Internet]. (2021 September). Volume 8, Issue 9, ofab453. Available from: https://doi.org/10.1093/ofid/ofab453
39. Rabaan, A.A. et al. Viral Dynamics and real-time RT-PCR CT values correlation with disease severity in COVID-19, Diagnostics (Basel, Switzerland). MDPI. [Internet]. (2021). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8232180/
40. AlBahrani, S., Alghamdi, M., Zakary, N. et al. Initial viral cycle threshold values in patients with COVID-19 and their clinical significance. Eur J Med Res [Internet]. (2022). 27, 101. Available from: https://doi.org/10.1186/s40001-022-00729-5
41. Camargo JF, Lin RY, Komanduri KV. Lack of correlation between the SARS-CoV-2 cycle threshold (Ct) value and clinical outcomes in patients with COVID-19. J Med Virol. [Internet]. 2021;93:6059–62. Available from: https://doi.org/10.1002/jmv.27171.
42. Yilmaz A, Marklund E, Andersson M, et al. Upper respiratory tract levels of severe acute respiratory syndrome coronavirus 2 RNA and duration of viral RNA shedding do not differ between patients with mild and severe/critical coronavirus disease 2019. J Infect Dis [Internet]. 2021; 23:15–18.
43. Argyropoulos KV, Serrano A, Hu J, et al. Association of initial viral load in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) patients with outcome and symptoms. Am J Pathol 2020; 190:1881–7.
44. Rhoads D, Peaper DR, She RC, et al. College of American Pathologists (CAP) Microbiology Committee Perspective: Caution Must Be Used in Interpreting the Cycle Threshold (Ct) Value. Clin Infect Dis. [Internet]. (2020). Available from: https://doi.org/10.1093/cid/ciaa1199.
45. Zheng A, Fan J, Yu F, et al. Viral load dynamics and disease severity in patients infected with SARS-CoV-2 in Zhejang province, China, January–March 2020: retrospective cohort study. BMJ [Internet]. 2020; 369:m144