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Association of SARS-COV-2 Cycle Threshold Values with Clinical and Epidemiological Features of Children and Adolescents in Iran

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Abstract

Background: The associations between the epidemiological, clinical, and serological features of coronavirus disease 2019 (COVID-19) and the nasopharyngeal viral load have not, yet, been understood completely.

Methods: This cross-sectional single center study of outpatient children and adolescents was conducted between January and March 2021. Reverse transcriptase polymerase chain reaction (RT-PCR) of nasopharyngeal swab specimens was positive for SARS-CoV-2. Nasopharyngeal Cycle threshold (Ct) values were measured for all patients considering different clinical features, age, and sex, in presence of covid-19 specific serum antibody.

Results: The data of 70 individuals with confirmed COVID-19 were analyzed (mean (range) age: 9.6 (5-14) years; 29 females (41%)). Sixty-four children (91.4%) were symptomatic at the time of sampling (mean time of symptom onset, 3.9 days). There were no differences in mean Ct values between the symptomatic and asymptomatic patients (31.4 vs 28.8, p=0.247). Ct values were significantly lower in cases with diarrhea (p=0.044) and younger children (p=0.003). No correlation was found between Ct values and gender (p=0.415). Serum antibody was measured in 25 (36%) patients. Presence of antibody was not associated with Ct values (p=0.121). Fifty-nine cases (84.3%) reported exposure to a SARS-CoV-2 positive household.

Conclusions: Higher nasopharyngeal Ct values, suggesting lower virus load, are related to older age, but there is no difference in Ct values between genders. Considering that diarrhea may predict lower Ct values in the respiratory system, the importance of early quarantine of children with atypical symptoms (such as gastrointestinal symptoms) or children in contact with a confirmed COVID-19 family member is highlighted.

Key Words: Children, Covid-19, Cycle threshold, Diarrhea, Sars-Cov-2, Viral load.

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1- INTRODUCTION

Following the COVID-19 pandemic which was declared by World Health Organization (WHO) on March 2020 (1), by August 2021 over 207 173 086 confirmed cases and 4 361 996 deaths had been announced worldwide (2). In December 2019, a novel coronavirus was named SARS-CoV-2, and was introduced as the etiology of COVID-19. By September 2021, over 5 million confirmed SARS-CoV-2 infected children have been reported in the US since the onset of the pandemic (3). Reverse transcriptase polymerase chain reaction (RT-PCR) is the gold standard diagnostic test for COVID-19 (4).

Although it appears that most of the COVID-19 cases among children are mild to moderate (5–7), there are several studies reporting that children can play a role in the transmission of COVID-19 among other children and adults (8–11). Some studies revealed that higher SARS-CoV-2 viral load in the respiratory tract was associated with a higher possibility of transmission (12, 13). The association between viral load with symptomatic and asymptomatic COVID-19 patients was studied previously (14, 15), while there are few studies investigating the correlation between nasopharyngeal viral load and specific signs/symptoms (16).

We hypothesized that age, sex, clinical features, serum antibody, and time since symptom onset are correlated to Ct values and, by extension, viral loads. The aim of this study was to investigate the association of SARS-COV-2 cycle threshold value with clinical and epidemiological features of children and adolescents in Iran, through a cross-sectional study.

2- MATERIALS AND METHODS

In this cross-sectional, single center study, nasopharyngeal swab specimens were collected from 70 outpatients at the Noor laboratory, Tehran, Iran. We collected plasma and serum from 25 (36%) patients to measure SARS-CoV-2 specific monoclonal human immunoglobulin M (IgM). RT-PCR cycle threshold (Ct) values were measured for all patients. Ct values less than 40 were considered positive, as reported in previous studies (17). Data including demographic features (i.e., age and sex), clinical characteristics (ie, fever, cough, shortness of breath, body aches, loss of smell or taste, and diarrhea), antibody (IgM) detection, SARS-CoV-2 exposure history and the time from any symptom of the disease onset to the positive test were recorded in a pre-designed checklist.

2-1. Inclusion and exclusion criteria

The inclusion criteria encompassed children and teenagers aged 5 to 14 years old, diagnosed with COVID-19 by RT-PCR between January and March 2021. The study sample was selected based on a census of COVID-19 positive children in the period of three months. The protocol of the present study has been registered at the ethics committee of Baqiyatallah University of Medical Sciences, Tehran, Iran. Exclusion criteria included subjects with underlying disease (such as respiratory and cardiovascular disease, diabetes, hypertension, dyslipidemia, obesity and dysmetabolic disorders), severe COVID-19 (SpO2 <93% or respiratory distress) or previously confirmed with COVID-19. All the subjects' parents or legal guardians signed an informed consent form.

2-2. Data analysis

Statistical analyses were conducted with SPSS software version 26 (IBM Inc). Continuous variables were expressed as means and standard deviations (SD). Categorical variables were presented as absolute numbers and percentages. The potential associations between Ct values and categorical variables or continuous

variables were analyzed through Mann-Whitney U, independent t test, and spearman rank correlation coefficients according to the normality tested with Shapiro-Wilk. Confidence intervals (CI) of 95% were used to evaluate any significant difference in Ct values between the variables. A p-value of less than 0.05 was considered as statistically significant.

3- RESULTS

A total of 70 outpatients, 29 (41%) females and 41 (59%) males with a mean age of 9.6 ± 2.2 years (ranged from 5 to 14 years) were included after being tested positive for SARS-CoV-2 RT-PCR on nasopharyngeal swab specimens. The majority of tested patients (64 (91.4%)) presented at least one known COVID-19 symptom at the time of collecting specimens. Fever (44.3%) was the most frequent symptom among them. Cough, diarrhea and body pain were other common symptoms (**Table 1**).

Table-1: Clinical and serological characteristics of children who tested positive for COVID-19

Characteristics		N (%)	Mean CT value	P-value
Symptomatic during sampling	Yes	64(91.4)	31.42	0.404 [a]
	No	6(8.6)	28.83	
Fever	Yes	31(44.3)	31.19	0.991 [a]
	No	39(55.7)	31.21	
Cough	Yes	18(25.7)	31.56	0.692 [a]
	No	52(74.3)	31.08	
Diarrhea	Yes	15(21.4)	29.20	0.044 [a]
	No	55(78.6)	31.75	
Body ache	Yes	14(20)	30.21	0.349 [a]
	No	56(80)	31.45	
Olfactory dysfunction	Yes	11(15.7)	32.09	0.465 [a]
	No	59(84.3)	31.03	
IgM	Yes	7(10.0)	28.71	0.121 [a]
	No	18(25.7)	31.67	
Shortness of breath	Yes	4(5.7)	30.25	0.559 [b]
	No	66(94.3)	31.26	
Respiratory symptoms (shortness of breath or cough)	Yes	18(25.7)	31.56	0.692 [a]
	No	52(74.3)	31.08	

Abbreviation: IgM, Immunoglobulin M
[a] Independent t-test
[b] Mann-Whitney U test

There was no significant difference regarding mean Ct values between males and females (31.56 in males and 30.69 in females, p=0.415). However, younger ages were significantly correlated with lower Ct values (p=0.003). Ct values were not significantly different between symptomatic and asymptomatic patients (p=0.247). Ct values were not related to the number of symptoms a patient presented (p=0.559). Mean Ct values were significantly lower between subjects presented with diarrhea (p=0.044). No correlation between age and diarrhea was reported (p=0.718). The mean (SD) of 3.9 ± 1.5 days were demonstrated between symptom onset and positive test. No significant relationship was found between

Ct values and the time from symptom onset to positive test (p=0.528). There was no significant difference (p=0.756) regarding Ct values between the patients with and without respiratory symptoms (such as cough or shortness of breath). Ct values and clinical features were not significantly different in IgM positive patients (p=0.121). Most of the children and adolescents confirmed with COVID-19 had been in contact with a SARS-CoV-2 positive household during the past two weeks prior sampling (59 (84.3%)).

4- DISCUSSION

Ct value, which is the number of cycles the PCR test takes to amplify the target gene in order to detect it, appears to be inversely related to viral load (18). Ct value as a predictive factor for viral load, may present adequate information about clinical and epidemiological features of SARS-COV-2 related to possible infectiousness (14).

Considering the fewer number of asymptomatic patients (n=6) compared to symptomatic ones (n=64) in our study, mean Ct values were similar among samples from symptomatic and asymptomatic individuals. In line with the present study, Aykac et al. (19) found no correlation between Ct values and symptom statuses which totally contradicts previous studies (15, 20) reporting higher viral loads in symptomatic children than in asymptomatic children.

In agreement with previous studies (19), we found similar viral loads in different gender groups. In the present study we found a positive correlation between age and Ct values which is in contrast to previous studies on large pediatric populations (19,21) reporting no Ct value-differences between age groups. In (22), Yonker et al. suggested that COVID-19 infected children of all ages may carry high levels of SARS-CoV-2.

We found no correlation between Ct values and time since symptom onset. This finding is consistent with some adult studies (23), in which similar to our study, the median time since symptom onset to sampling was 4 days. While another study of 256 pediatric individuals reported significantly lower Ct values within 2 days after symptom onset (24). Another study on 123 SARS-CoV-2 positive children showed that longer time since symptom onset was correlated to higher Ct values (25). In our study 54 individuals (80%) were presented to the laboratory more than 2 days after symptom onset. Consequently, our study may have missed the time in which the Ct values are lower.

Our study showed that most (84%) of the infected children were in contact with a SARS-CoV-2 positive household, which is consistent with the findings of a previous study (25) by Chung et al. showing that 80% of children were in contact with at least one positive individual, and most contacts (68%) were in the same household.

Our data showed lower Ct values in nasopharyngeal samples, corresponding to higher viral loads, in cases with diarrhea, while Balajelini et al. (16) found no correlation between nasopharyngeal viral load with diarrhea but with olfactory and gustatory dysfunction. In terms of fecal samples, a meta-analysis of 4,243 COVID-19 patients revealed that patients presented with diarrhea have higher stool viral loads than those without diarrhea (26). Previous studies on patients with SARS in 2004 reported that nasopharyngeal viral load had been significantly higher in patients with diarrhea (27); whereas, there is no such result about SARS-CoV-2 infected patients. In our assessment, diarrhea was not related to age. A previous meta-analysis (28) found that children older than 5 years were more likely to be presented with diarrhea. Our study was performed on children older than 5 years of age.

Present investigations have demonstrated that the mean Ct values of nasopharyngeal specimens are similar among patients with positive serum IgM. Additionally, in a study by Li et al. (29), no association was found between nasopharyngeal viral load and anti-N IgM levels.

4-1. Limitations of the study

We acknowledge that our investigation has some limitations. This study was conducted through the emergence of new SARS-CoV-2 variants and there is uncertainty about the impact that new variants could have had on our findings. Sample size was relatively small, especially in the asymptomatic subgroup, which might have led to selection bias or some significant associations not being detected. We did not conduct any culture to detect viable viruses; therefore, the inverse association of Ct value and viral load is according to the previous studies (30). Early specimen-sampling soon after symptom onset was limited. Due to our cross-sectional study design, this investigation was not powered to detect the correlation between Ct values and transmission risk of covid-19.

5- CONCLUSION

Overall, our investigation suggests that younger age and presence of specific symptoms including diarrhea may predict more viral load in the respiratory system. Mean Ct values were similar between the symptomatic and asymptomatic individuals and between the genders. These findings demonstrate the importance of rapid quarantine of patients who have been in close contacts with confirmed cases and have atypical COVID-19 symptoms such as diarrhea. Ultimately, further epidemiological studies are necessary to clarify the viral load correlation with transmission potential and disease severity of COVID-19 infected children and adolescents.

6- ETHICAL CONSIDERATIONS

The protocol of the present study has been registered at the ethics committee of Baqiyatallah University of Medical Sciences, Tehran, Iran.

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