Original Article

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Pediatric Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2): Clinical Characteristics and Cycle Threshold Value (CT value) of Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) of Nasopharyngeal Samples

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Abstract

Introduction: We aimed to characterize epidemiological and clinical characteristics of children and adolescents with Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection, and to evaluate relationship of cycle threshold value (CT value) of Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) test (As surrogate marker of viral load) with patient age and severity of infection.

Methods: We retrospectively collected data of children and adolescents admitted in our center from April 2020 to July 2020 with positive RT-PCR test for SARS-CoV-2.

Results: Total 62 children, with median (IQR) of age 96 (54 - 122) months and 39 adolescents with median (IQR) of age 19.5 (18.2 - 20) years were included. 56 (90%) children and 34 (87%) adolescents had history of SARS-CoV-2 positive cases in their family. Only nine (14%) children had associated risk factor for severe SARS-CoV-2 infection. Fever was the commonest symptom which was present in 24 (39%) children and 16 (41%) adolescents. Cough was present in 17 (27%) children and 10 (26%) adolescents. Diarrhea was found in 14 (23%) children and three (8%) adolescents. CT values of RT-PCR test were similar in children and adolescence (p = 0.48). However, asymptomatic children had higher CT values than symptomatic children (p = 0.01).

Conclusions: Majority of children have asymptomatic or mild SARS-CoV-2 infection with similar CT values in children and adolescents.

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Introduction

It has been more than two years since outbreak of Corona virus disease 2019 (COVID-19) caused by severe acute respiratory syndrome corona virus 2 (SARS-CoV-2).[1] Globally studies have shown that primary and secondary attacks rate of SARS-CoV-2 in children are same as in adults population but children were rarely index cases in household, and adult age group patients are often the index cases in household who had travelled or moved out in public places.[2]

There are limited studies, as such from India, about epidemiology, clinical manifestations and risk of infectivity of SARS-CoV-2 in different age groups of children. Moreover, there has also been association between viral load and risk of transmission, as seen in other similar respiratory diseases.[3-5] There is uncertainty about the extent to which children can be source of infection as Indian adult citizens are being immunized, with the largest vaccination program of the world.[6]

Hence, we aimed to study the epidemiology, clinical characteristics and viral loads in nasopharyngeal swabs as a surrogate marker of infectivity in different age groups of children. We assessed viral loads by measuring cycle threshold value (CT value) of Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) test for SARS-CoV-2 in nasopharyngeal sample of COVID-19 patients. The CT value is the number of replications cycle required for RT-PCT test to become positive.

Methods

We did retrospective review of 101 patients' (Children between one month to 15 years and adolescents between 15 years to 20 years) admitted from 01 April 2020 to 31 July 2020 in a dedicated tertiary care COVID-19 center in Maharashtra, India. The study was approved by the Institute Ethics Committee. COVID-19 case was identified by detection of nucleic acid of SARS-COV-2 in nasopharyngeal swab using RT-PCR test. Details of each admitted cases including demographic data, contact and travel history, vaccination history, living condition, clinical manifestation, co-morbid condition, treatment received and outcome were entered in pre-designed proforma. Children were classified as asymptomatic infection who had positive RT-PCR test without any clinical features, upper respiratory tract infection when there was fever, cough, nasal congestion, etc without signs of pneumonia in chest radiology, mild COVID-19 pneumonia with respiratory symptoms and radiological evidence whereas severe COVID-19 pneumonia was defined in presence of fast breathing, hypoxemia (SpO_2 < 90%), altered sensorium, feeding difficulty.[7] Critical infection had any one of following: respiratory failure, shock or multiple organ failure.[8] Sample for RT-PCR were taken from nasopharyngeal swab with AllplexTM 2019-nCoV Assay by Seegene Inc. CT value represents the number of replication cycles required for detection of virus. It is inversely correlated to amount of viral nucleic acid in sample. Sample was considered positive when CT value of all genes were less than 40 cycles. In case of repeat RT-PCR testing, we included only initial RT-PCR test CT value for this study. All data were analyzed by using STATA / IC statistical software version 16.0 (StataCorp). The quantitative variables of different groups were measured in median with interquartile ranges and compared using Krushal-wallis test, whereas all categorical data were analyzed by chi ^{2} test. We used spearman correlation test to evaluate relationship between two variables. A P value of < 0.05 was considered statistically significant.

Results

A total of 101 patients were enrolled in this study. The demographic, clinical characteristics and CT value and clinical severity have been depicted respectively in Tables 1, 2 and 3 and Figure 1.

Table 1. Clinical characteristics of the study population

Variable		Children less than 15 years of age (n = 62)	Adolescents age (> 15 years to 20 years), n = 39	P value
Presence of risk factor, n (%)		9 (7+2) (14%)	4 (10%)	P = 0.54
Fever, n (%)		24 (39%)	16 (41%)	P = 0.81
Cough, n (%)		17 (27%)	10 (26%)	P = 0.84
Running nose, n (%)		10 (16%)	6 (15%)	P = 0.92
Sore throat, n (%)		11 (18%)	9 (23%)	P = 0.51
Body ache, n (%)		4 (6%)	6 (15%)	P = 0.15
Loss of appetite, n (%)		3 (5%)	5 (13%)	P = 0.25
Diarrhea, n (%)		14 (23 %)	3 (8%)	P = 0.04
Loss of smell, n (%)		8 (13%)	6 (15%)	P = 0.62
Loss of taste, n (%)		4 (6%)	5 (13%)	P = 0.26
Symptoms category	Asymptomatic, n (%)	34 (55%)	22 (57%)	P = 0.17
	Mild, n (%)	28 (45%)	15 (38%)	
	Moderate, n (%)	0	2 (5%)	

Table 2. Demographic characteristics of the study population

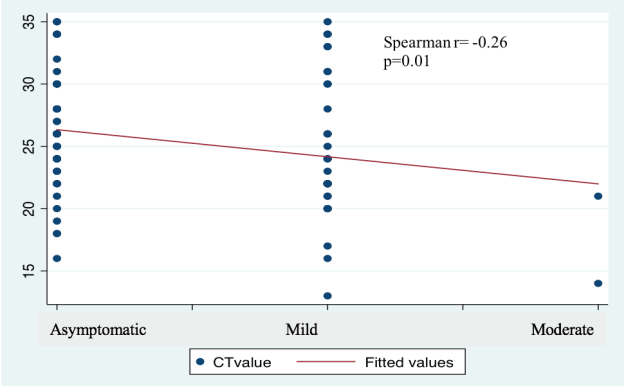
		Children less than 15 years of age (n = 62)	Adolescents age (> 15 years to 20 years), (n = 39)	P value
BMI (Kg / m^2), median (IQR)		15.6 (14.0, 17.7)	20.2 (19.1, 21.2)	P = 0.001
CT value, median (IQR)		25.5 (22, 30)	24 (22, 28)	P = 0.59
Male, n (%)		34 (55%)	20 (51%)	P = 0.75
Residence	Rural, n (%)	18 (29%)	3 (8%)	P = 0.01
	Urban, n (%)	44 (71%)	36 (92%)	
Father occupation	Govt. sec., n (%)	47 (76%)	22 (56%)	P = 0.002
	Agriculture, n (%)	10 (16%)	3 (8%)	
	Private sec., n (%)	5 (8%)	14 (36%)	
(Size of family (numbers	Three , n (%)	12 (19%)	12 (31%)	P = 0.21
	four, n (%)	36 (58%)	16 (41%)	
	five, n (%)	8 (13%)	9 (23%)	
	six, n (%)	2 (3%)	2 (5%)	
Vaccination received	BCG + MMR, n (%)	40 (65%)	34 (88%)	P = 0.06
	BCG+MMR+ Pneumococcal, n (%)	11 (18%)	1 (2%)	
	BCG+MMR+ Pneumococcal+ Influenza, n (%)	4 (6%)	1 (2%)	
	Only BCG , n (%)	7 (11%)	3 (8%)	
Travel history present, n (%)		4 (6%)	4 (10%)	P = 0.40
Family member infected, n (%)		56 (90%)	34 (87%)	P = 0.30
Infected member in family	Father alone, n (%)	22 (35%)	21 (54%)	P = 0.15
	Mother alone, n (%)	4 (6%)	4 (10%)	
	Sibling alone, n (%)	12(19%)	7 (18%)	
	Both parent, n (%)	16 (26%)	7 (18%)	
	All family members, n (%)	7 (11%)	0	

BMI-body mass index, CT value – cycle threshold value, BCG- Bacille Calmette-Guérin, MMR-measles, mumps, rubella.

Table 3. Severity of symptoms and cycle threshold (CT) values

Severity of symptoms	CT values (median , IQR)	P Value
Asymptomatic (n = 56)	26 (24, 28)	0.01
Mild (n = 43)	22 (22, 30)	
Moderate (n = 2)	17.5 (14, 21)	
Severe (n = 0)		

Figure 1. Severity of symptoms and cycle threshold (CT) values



We did not notice any statistically significant correlation between severity of infection and vaccination received or presence of risk factors of COVID-19 infection either. We noted that CT value had weak negative but statistically significant correlation with severity of COVID infection (Table 3, Fig 1).

Discussion

Since COVID-19 is a novel infection, there is limited knowledge about it, especially from paediatric age group. To date, majority of COVID-19 related paediatric studies have originated from either China or western countries, data from Indian subcontinent are scarce.[9,10] As many country specific factors (climate, food habit, socio-economic status and health care facilities etc) may affect natural course of any pandemic, we reported an observation study of epidemiology, clinical characteristics and CT value of RT-PCR (as surrogate marker of viral load) in nasopharyngeal swab of SARS-CoV-2 infected children from a large city in southern part of India. It was one of the epicenters of COVID-19 cases in India.[11]

There was male predominance (55%) in admitted children in our study which is almost similar to another large study from China.[8] As seen in previous studies, main source of infection in majority of children was house hold contact.[12,13] This was prominently seen in our study too. Clinically, almost all children in this study had

asymptomatic or mild SARS-CoV-2 infection. Previous multicenter studies and recent meta-analysis too, revealed that majority of SARS-CoV-2 infected children had mild or no symptoms of infection worldwide.[12,14] Fever (39%), cough (27%) and diarrhea (23%) were common symptoms in children in our study. Similar clinical characteristics were reported in cohorts of previous large studies from China too.[8,9] We noticed that higher proportion of children had symptomatic infection with increasing age. Complaints of loss of taste, loss of appetite and body ache were more prevalent in > 15 years of adolescence than children, whereas diarrhea was more common in children (23% vs 8%) in our study. Similar results were reported in another multicenter study including tertiary centers of China, France and Germany.[15] However, we found that higher proportion of children in our study had diarrheal episodes compared to previous studies from US and China.[16,17] The reason for this differences may be due to sanitation, food habits, vaccination coverage, and poor nutritional status. We did not have any child with severe or critical infection, which is also supported by finding of the COVID-19 data summary-NYC Heath, in which less than 1% children had critical infection. [18]

Besides diagnosing, it is also important to know whether infected person is infectious or not, for containing pandemic. Though, viral cell culture is gold standard to determine infectivity but in the absence of it, one may use viral load / CT value of RT- PCR as a surrogate marker of likelihood of infectivity. The correlation between CT values of RT-PCR test and viral cell culture was found significant in a study in France.[19] Similarly, another study from Canada showed there was significant correlation between CT values and infectivity in cell culture; and concluded that infectivity of patients may be low who has CT value >24.[20] However, a study from US showed positive culture growth from samples with CT values of 34.[21] Later, systematic review from Stanford University analyzed this study and revealed that study population (elderly patients in elder care facility) of this study might not represent the general population.[22] Recently, authors of another systematic review including 17 studies on viral load (CT values) and symptom severity, concluded that infectivity decreases after a week of viral shedding and cycle threshold value of 24.[23] In our study, we did not find any significant difference in CT values of paediatric and adolescence age groups.

Since the early phase of this pandemic, many studies worldwide assessed relationship between severity of infection, age and infectivity. Earlier this year, a study from Chicago, U.S. including 97 children and 48 adults revealed similar CT values in older children (> 5 years to 17 years of age) and adults. However, younger children (< 5 years of age) had significant lower CT values than adults.[24] In another study from Switzerland, authors compared viral load values of 53 children with values of 352 adults and found no significant difference.[25] Similarly, a study of 201 children from Greece reported no difference in viral load of paediatric and adolescence age groups, neither any difference of viral loads between symptomatic and asymptomatic children.[26] However, another study from US comprising of 339 asymptomatic and 478 symptomatic children found significantly higher CT values of asymptomatic children than symptomatic children of all age groups.[27] We too found significant higher median (IQR) CT values of asymptomatic children than mild symptomatic children, 26 (24, 28) vs 22 (22, 30), p = 0.01.

Our study has some limitations. Asymptomatic children in our study may not be representing recent infection due to possibility of picking up remote infection in the screening tests. However, the strength of our study is that the nasopharyngeal swabs were taken by single team of trained health care staff and analyzed by same commercial kit in microbiological laboratory of our center.

Conclusions

To conclude, majority of children have asymptomatic or mild SARS-CoV-2 infection with similar CT values in children and adolescents. Further studies are needed to evaluate the role of children in the ongoing pandemic with upcoming new mutations.

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