Viral shedding and immunological features of children COVID-19 patients

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Abstract

Background SARS-CoV-2 could infect people at all ages, and the viral shedding and immunological features of children COVID-19 patients were analyzed.

Methods Epidemiological information and clinical data were collected from 35 children patients. Viral RNAs in respiratory and fecal samples were detected. Plasma of 11 patients were collected and measured for 48 cytokines.

Results 40% (14/35) of the children COVID-19 patients showed asymptomatic infections, while pneumonia shown by CT scan occurred in most of the cases (32/35, 91.43%). Elevated LDH, AST, CRP, neutropenia, leukopenia, lymphopenia and thrombocytopenia occurred in some cases, and CD4 and CD8 counts were normal. A total of 22 cytokines were significantly higher than the healthy control, and IP-10, IFN-α2 of them in children were significantly lower than the adult patients. Meanwhile, MCP-3, HGF, MIP-1α, and IL-1ra were similar or lower than healthy control, while significantly lower than adult patients. Viral RNAs were detected as early as the first day after illness onset (d.a.o) in both the respiratory and fecal samples. Viral RNAs decreased as the disease progression and mostly became negative in respiratory samples within 18 d.a.o, while maintained relatively stable during the disease progression and still detectable in some cases during 36~42 d.a.o.

Conclusion COVID-19 in children was mild, and asymptomatic infection was common. Immune responses were relatively normal in children COVID-19 patients. Cytokine storm also occurred in children patients, while much weaker than adult patients. Positive rate of viral RNAs in fecal samples was high, and profile of viral shedding were different between respiratory and gastrointestinal tract.

Background

Coronavirus Disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was firstly reported in late December, 2019 [1, 2], and rapidly caused the ongoing pandemic [3]. As of Apr. 24, 2020, a total of 2,549,632 cases with 175,825 fatal cases were reported all around the world [3]. Although majority of the COVID-19 patients were adults, children patients were also found with a small portion [4–9]. Recently, some studies have revealed the clinical and epidemiological characteristics of children COVID-19 cases, and found that children patients mostly show mild respiratory symptoms and even asymptomatic infection [7, 8, 10].

Viral shedding varies in different viruses and ages, and elucidation of the viral shedding profile is crucial for the laboratory diagnosis, treatment and control of COVID-19. Recent studies have elucidated the viral shedding patterns of SARS-CoV-2 mainly based on adult patients [11–14]. Cytokine storm, which contributes to acute lung injury and development of acute respiratory distress syndrome (ARDS) [15], has been found during SARS-CoV-2 infection [16–18]. Meanwhile, several cytokines have been shown to closely associate with the diseases severity and outcome of COVID-19 in adults [18]. However, whether the cytokine storm occurred in children patients is also currently unclear. In this study, we analyzed the

viral shedding and immunological features of children COVID-19 patients based on a case series of 35 laboratory-confirmed children COVID-19 patients.

Methods

Patient information and data collection

A total of 35 children COVID-19 patients hospitalized in our hospital were included for the analyses. 50 adult COVID-19 patients and 8 healthy controls reported elsewhere were also included for comparison [18]. Clinical information and laboratory result were collected at the earliest time-point after hospitalization. The study protocol was approved by the Ethics Committees of Shenzhen Third People's Hospital. Informed consents were obtained from all patients or patients family members. The study was conducted in accordance with the International Conference on Harmonisation Guidelines for Good Clinical Practice and the Declaration of Helsinki and institutional ethics guidelines.

Quantitative Reverse-transcriptase Polymerase Chain Reaction (qrt-pcr)

qRT-PCR were done as previously reported [18, 19]. In brief, throat swabs, nasal swabs, sputum and rectal swabs were collected from children COVID-19 patients upon admission and thereafter. Viral RNAs were extracted from clinical specimens using the QIAamp RNA Viral Kit (Qiagen, Heiden, Germany). A China Food and Drug Administration (CDFA) approved commercially kit (BioGerm Co., Shanghai, China) were used for the detection of SARS-CoV-2 specific RNAs. Samples with a cycle threshold (Ct) value ≤ 37.0 were considered putatively positive. Samples whose Ct was higher than 37 were re-tested and considered positive if Ct was ≥ 37 but ≤ 40 and negative if viral RNAs were undetectable on the second test.

Cytokine And Chemokine Measurements

The plasma of the children COVID-19 patients (N = 11) were collected at the earliest possible time-point after hospitalization. The concentrations of 48 cytokines and chemokines were measured using Bio-Plex Pro Human Cytokine Screening Panel (Bio-Rad) as previously reported together with plasma collected from 50 adult patients and 8 healthy control [17, 18].

Disease Severity Classification Of Children Covid-19 Patients

Disease severity classification were evaluated as previously reported [17, 19], except that the criterion of severe COVID-19 for children is defined as following: 1) respiratory distress with the exclusion of cry and fever (age specific reference ranges: < 2 months: respiration rate (RR) ≥ 60/min; 2 ~ 12 months: RR ≥ 50/min; > 5 years: RR ≥ 30/min), 2) resting oxygen saturation ≤ 92%, or 3) Assisted respiration, cyanosis or intermittent apnea, 4) somnolence, convulsions, 5) Refusing to eat or difficult to feed, symptoms of dehydration.

Statistical analysis

Fisher exact test was used to compare the indicated rates between the symptomatic and asymptomatic cases. Mann-Whitney U test was used to compare plasma cytokine levels between two groups. All statistical tests were calculated using Graphpad Prism 7.0 for Windows. P value less than 0.05 was considered statistically significant.

Results

Epidemiological and clinical characteristics of children COVID-19 patients.

A total of 35 children COVID-19 patients were included in our study. The median age is 7.5 with a range of 1.5 to 17 years-old, and 13 (37.14%) were male. Two case possessed chronic respiratory diseases, one is asthma and the other pharyngitis. Cough (41.94%) was the most common initial symptoms, followed by fever (38.71%). Expectoration, headache, chill and diarrhea also occasionally happened in some cases. Of note, 40% the cases showed asymptomatic infections. 32 (93.55%) children's family possessed at least 1 infected family member, while three children were the only COVID-19 cases in their family. 26 children were reported to be either living in Wuhan or having family members who visited Wuhan/Hubei. The median days of onset to admission and duration of hospitalization is 0 and 17, respectively. Viral RNAs were detected in fecal samples from 17 (48.57%) cases. Pneumonia occurred in most of the cases (32/35, 91.43%), and hepatic insufficiency also occurred in some cases (5/35, 14.29%). Of all the 35 cases, 32 cases were moderate, and 3 were mild cases. 34 patients received antiviral treatment within 5 d.a.o, and 85.71% (30/35) of the children received antiviral treatment within 2 d.a.o (Table S1). The combination of interferon alpha and lopinavir was the most frequently used antiviral treatment, and no patients received gamma globulin, corticosteroid or mechanical ventilation treatments. All the patients discharged from hospital (Table S1).

Laboratory and CT findings of children COVID-19 patients.

A complete blood count with differential was assessed for each patient either on the date of hospital admission, or at the earliest time-point after admission (Table 2). Elevated lactate dehydrogenase (LDH), aspartate amino transferase (AST), C-reactive protein (CRP), neutropenia, leukopenia, lymphopenia and thrombocytopenia occurred in some cases with different rates. CT scan of the children patients showed ground-glass, hazy patchy shadows or high density shadows, mainly in the peripheral part of the lung (Figure S1). Moreover, 57.14% of patients showed bilateral involvement of the lung, and 34.29% showed unilateral involvement. Furthermore, based on the initial symptoms, the cases were divided into symptomatic (n = 21) and asymptomatic (n = 14) groups. Elevated LDH, AST, CRP, neutropenia, leukopenia, lymphopenia as well as unilateral and bilateral involvement of lung were also found in asymptomatic cases, with no statistic differences when compared to the symptomatic cases. Except for CPR which was higher in symptomatic cases, the other indexes of complete blood count were comparable between the two groups.

Table 2
Laboratory and chest radiography findings in children patients of COVID-19 upon admission.

Variables	Children COVID-19 cases			P values
	Total (N = 35)	Symptomatic (N = 21)	Asymptomatic (N = 14)	
WBC ($\times 10^9$/L)†	5.11 (4.60, 7.79)	5.11 (4.63, 7.67)	5.3 (4.37, 7.97)	0.724
LYM ($\times 10^9$/L)†	2.52 (1.99, 3.27)	2.49 (1.83, 3.65)	2.6 (2.18, 2.98)	0.987
NEU ($\times 10^9$/L)†	2.12 (1.47, 3.53)	2.25 (1.65, 3.32)	2.05 (1.35, 3.64)	0.449
PLT ($\times 10^9$/L)†	253 (222, 298)	243 (205.5, 309)	258 (234.75, 299.5)	0.490
AST (U/L)†	35 (23, 42)	35 (20.5, 42.8)	36.45 (22.5, 43.75)	0.686
ALT (U/L)†	15.9 (11, 24.5)	16 (11.95, 25.75)	13.95 (8.93, 24.28)	0.533
TB (umol/L)†	8.6 (6.1, 11)	8.8 (5.85, 10.75)	6.9 (5.65, 11.90)	0.544
ALB (g/L)†	45.1 (43.5, 46.8)	45.6 (43.5, 47.25)	44.4 (43.6, 45.73)	0.273
CRE (μmol/L)†	35 (28, 45)	35 (26, 48.5)	31 (28.9, 44.50)	0.887
CK (U/L)†	83 (68.63, 94.5)	91.5 (84, 100.25)	71 (65.25, 92)	0.113
LDH (U/L)†	261 (198, 496.5)	266 (224, 516)	218 (187, 453)	0.366
CRP (mg/L)†	2.02 (0.52, 5.26)	4.33 (1.14, 7.85)	0.75 (0.32, 2.26)	0.047
PCT (ng/mL)†	0.05 (0.03, 0.08)	0.06 (0.03, 0.10)	0.05 (0.01, 0.06)	0.187
CD4 (count/μl)†	781 (684, 931.5)	857.5 (704.8, 1029.3)	707 (646, 954)	0.495

† Values shown represent the mean and inter-quartile range (IQR).

Abbreviations:

WBC: white blood cell; LYM: lymphocyte; NEU: neutrophil; PLT: platelet; AST: aspartate aminotransferase; ALT: alanine aminotransferase; CRE: serum creatinine; CK: creatine kinase; CRP: C-reactive protein; LDH: lactate dehydrogenase.

Age specific reference ranges used to define abnormalities in blood parameters:

Leukopenia ($\times 10^9$/L): 2 months-2 years: <5, > 2 years: <4; Lymphopenia ($\times 10^9$/L): 2−11 months: <4.0, 1−11 years: <1.5, 12 + years: <1; Neutropenia ($\times 10^9$/L): All ages: <1.5; Neutrophilia ($\times 10^9$/L): All ages: >8.5; Thrombocytopenia ($\times 10^9$/L): All ages: <150; Elevated AST(U/L): All ages: >50; Elevated ALT (U/L): All ages: >50; Elevated CRE(μmol/L): All ages: >120; Elevated CK(U/L): All ages: >200; Elevated CRP (nmol/L): All ages: >10; Elevated LDH (U/L): <7 years: >400, 7−15 years: >300, 16 + years: >250.

Variables	Children COVID-19 cases			P values
	Total (N = 35)	Symptomatic (N = 21)	Asymptomatic (N = 14)	
CD8 (count/µl)†	600 (512, 922)	676 (391, 905)	600 (540, 1062)	0.558
CD4/CD8†	1.18 (1.06, 1.56)	1.30 (1.04, 1.58)	1.09 (1.05, 1.35)	0.495
Leukopenia	3/35 (8.57%)	1/21 (4.76%)	2/14 (14.29%)	0.324
Lymphopenia	2/35 (5.71%)	1/21 (4.76%)	1/14 (7.14%)	0.766
Neutropenia	8/35 (22.86%)	3/21 (14.29%)	5/14 (35.71%)	0.139
Neutrophilia	0/35 (0%)	0/21 (0%)	0/14 (0%)	1.000
Thrombocytopenia	2/35 (5.71%)	2/21 (8.33%)	0/14 (0%)	0.234
Elevated AST	5/35 (14.29%)	2/21 (8.33%)	3/14 (21.43%)	0.324
Elevated ALT	1/35 (2.86%)	1/21 (4.76%)	0/14 (0%)	0.407
Elevated CRE	0/35 (0%)	0/21 (0%)	0/14 (0%)	1.000
Elevated CK	0/35 (0%)	0/21 (0%)	0/14 (0%)	1.000
Elevated CRP	3/35 (8.57%)	3/21 (14.29%)	0/14 (0%)	0.139
Elevated LDH	12/35 (34.29%)	8/21 (38.10%)	4/14 (28.57%)	0.561
CT scan				
Normal	3/35 (8.57%)	3/21 (14.29%)	0/14 (0%)	0.139
Unilateral involvement	12/35 (34.29%)	6/21 (28.57%)	6/14 (42.86%)	0.383

† Values shown represent the mean and inter-quartile range (IQR).

Abbreviations:

WBC: white blood cell; LYM: lymphocyte; NEU: neutrophil; PLT: platelet; AST: aspartate aminotransferase; ALT: alanine aminotransferase; CRE: serum creatinine; CK: creatine kinase; CRP: C-reactive protein; LDH: lactate dehydrogenase.

Age specific reference ranges used to define abnormalities in blood parameters:

Leukopenia (× 10^9/L): 2 months-2 years: <5, > 2 years: <4; Lymphopenia (× 10^9/L): 2−11 months: <4.0, 1−11 years: <1.5, 12 + years: <1; Neutropenia (× 10^9/L): All ages: <1.5; Neutrophilia (× 10^9/L): All ages: >8.5; Thrombocytopenia (× 10^9/L): All ages: <150; Elevated AST(U/L): All ages: >50; Elevated ALT (U/L): All ages: >50; Elevated CRE(µmol/L): All ages: >120; Elevated CK(U/L): All ages: >200; Elevated CRP (nmol/L): All ages: >10; Elevated LDH (U/L): <7 years: >400, 7−15 years: >300, 16 + years: >250.

Variables	Children COVID-19 cases			P values
	Total (N = 35)	Symptomatic (N = 21)	Asymptomatic (N = 14)	
Bilateral involvement	20/35 (57.14%)	12/21 (57.14%)	8/14 (57.14%)	1.000
† Values shown represent the mean and inter-quartile range (IQR).				
Abbreviations:				
WBC: white blood cell; LYM: lymphocyte; NEU: neutrophil; PLT: platelet; AST: aspartate aminotransferase; ALT: alanine aminotransferase; CRE: serum creatinine; CK: creatine kinase; CRP: C-reactive protein; LDH: lactate dehydrogenase.				
Age specific reference ranges used to define abnormalities in blood parameters:				
Leukopenia ($\times 10^9$/L): 2 months-2 years: <5, > 2 years: <4; Lymphopenia ($\times 10^9$/L): 2−11 months: <4.0, 1−11 years: <1.5, 12 + years: <1; Neutropenia ($\times 10^9$/L): All ages: <1.5; Neutrophilia ($\times 10^9$/L): All ages: >8.5; Thrombocytopenia ($\times 10^9$/L): All ages: <150; Elevated AST(U/L): All ages: >50; Elevated ALT (U/L): All ages: >50; Elevated CRE(μmol/L): All ages: >120; Elevated CK(U/L): All ages: >200; Elevated CRP (nmol/L): All ages: >10; Elevated LDH (U/L): <7 years: >400, 7−15 years: >300, 16 + years: >250.				

Expression profiles of cytokines in children COVID-19 patients.

Then, we examined 48 cytokines in the plasma samples, and compared the expression levels with adult COVID-19 patients and healthy adult control. The results showed that 22 cytokines were significantly higher than the healthy control. Of the 22 cytokines, IP-10 and IFN-α2 in children were significantly lower than the adult patients, and the others comparable. Meanwhile, MCP-3, HGF, MIP-1α, IL-6, M-CSF, CTACK and IL-18 were similar with healthy control, while significantly lower than adult patients. IL-1ra was significantly elevated in adult patients, while it was lower than the healthy control in children.

Profile of viral shedding in children COVID-19 patients.

The viral loads were detected by qRT-PCR upon admission and thereafter. Daily measurements of viral load from respiratory and fecal specimens are summarized in Fig. 2, lower Ct values indicated higher viral loads. In respiratory samples, high viral loads were found as early as the first day after illness onset (d.a.o), and decreased as the disease progression. Most of patients became negative in respiratory samples within 18 d.a.o, and viral RNAs were re-detectable in two patients at 26, 28 and 33 d.a.o. A total of 17 patients were tested positive in the fecal samples. Viral RNA was also detected as early as the first d.a.o, while viral loads were relatively stable during the disease progression. Viral RNAs were still detectable during 36 ~ 42 d.a.o.

Discussion

Similar with previous study [7], only 37.14% of the children patients showed fever, while cough was most frequent symptoms with a rate of 48.57% (Table 1). A previous study showed that some children COVID-19 cases might display no symptoms or radiologic features of pneumonia, and some might have radiologic features of pneumonia but did not have any symptoms of infection [7]. Notably, 40% of the children cases in our study showed asymptomatic infection before admission (Table 1), however, CT scan of these children showed radiologic features of pneumonia (Table 2). In contrast, 3 of the symptomatic patients showed no radiologic features of pneumonia. All the asymptomatic children cases did the virological tests for investigation, as almost all the patients' family possessed at least one family member of laboratory confirmed COVID-19 patient. Family cluster of COVID-19 patients were frequently found [10, 20, 21], suggesting that it was easy to transmit among family members, and transmission from family members might serve as the main cause of infection for children. Asymptomatic adult patients were also found with lower rate [4−6], and these patients were also shown to be possible source of infection [22−25]. These asymptomatic patients posed a great threat to the control and prevention of COVID-19, as they were hard to be found unless family members developed symptoms and laboratory confirmed, which is of great concern during the control of COVID-19 pandemic. Meanwhile, there were no statistical differences in epidemiological information, CT scan, blood biochemical and complete blood count between symptomatic and asymptomatic patients (Tables 1 and 2), except a higher level of CRP was found in symptomatic patients, which might contribute to the fever of the symptomatic infection.

Table 1
Epidemiological and clinical features of children cases hospitalized with SARS-CoV-2 infections.

Characteristics	SARS-CoV-2 childhood cases			P value
	Total (N = 35)	Symptomatic (N = 21)	Asymptomatic (N = 14)	
Median age (range)	7 (1.5, 17)	8 (1.5, 17)	7 (1.5, 13)	0.390
Age subgroups				
0−1 year	0/35 (0%)	0/21 (0%)	0/14 (0%)	1.000
1−3 years	5/35 (14.29%)	3/21 (14.29%)	2/14 (14.29%)	1.000
3−6 years	8/35 (22.86%)	4/21 (19.05%)	4/14 (28.57%)	0.511
6 ~ 14 years	19/35 (54.23%)	11/21 (52.38%)	8/14 (51.74%)	0.782
14 ~ 18 years	3/35 (8.57%)	3/21 (14.29%)	0/14 (0%)	0.139
Male (%)	13/35 (37.14%)	9/21 (42.86%)	4/14 (28.57%)	0.392
BMI	17.1 (15.0, 19.9)	17.7 (15.3, 21.1)	16.3 (13.88, 18.08)	0.083
Co-existing chronic medical conditions				
Chronic heart disease	0/35 (0%)	0/21 (0%)	0/14 (0%)	1.000
Chronic lung disease	1/35 (2.86%)	0/21 (0%)	1/14 (7.14%)	0.214
Chronic renal disease	0/35 (0%)	0/21 (0%)	0/14 (0%)	1.000
Chronic liver disease	0/35 (0%)	0/21 (0%)	0/14 (0%)	1.000
Diabetes	0/35 (0%)	0/21 (0%)	0/14 (0%)	1.000
Cancer	0/35 (0%)	0/21 (0%)	0/14 (0%)	1.000
Initial symptoms				
Fever	13/35 (37.14%)	13/21 (61.90%)	0/14 (0%)	< 0.0001
Cough	16/35 (45.71%)	16/21 (76.19%)	0/14 (0%)	< 0.0001
Expectoration	1/35 (2.86%)	1/21 (4.76%)	0/14 (0%)	0.407
Headache	1/35 (2.86%)	1/21 (4.76%)	0/14 (0%)	0.407

NA: Not applicable.

Characteristics	SARS-CoV-2 childhood cases			*P* value
	Total (N = 35)	Symptomatic (N = 21)	Asymptomatic (N = 14)	
Myalgia	0/35 (0%)	0/21 (0%)	0/14 (0%)	1.000
Chill	2/35 (5.71%)	2/21 (9.52%)	0/14 (0%)	0.234
Nausea or vomiting	0/35 (0%)	0/21 (0%)	0/14 (0%)	1.000
Diarrhea	1/35 (2.86%)	1/21 (4.76%)	0/14 (0%)	0.407
Disease severity				
Severe	0/35 (0%)	0/21 (0%)	0/14 (0%)	1.000
Moderate	32/35(91.43%)	18/21 (85.71%)	14/14 (100%)	0.139
Mild	3/35(8.57%)	3/21 (14.29%)	0/14 (0%)	0.139
Exposure history				
Traveling history to Wuhan/Hubei	26/35 (74.29%)	17/21 (80.95%)	9/14 (64.29%)	0.269
Family cluster	32/35 (91.43%)	19/21 (90.48%)	13/14 (92.86%)	0.805
Anal swab positive of viral RNA	17/35 (48.57%)	5/12 (41.67%)	8/19 (42.11%)	1.000
Interval, median days (IQR)[+]				
Onset to admission	0 (0, 3)	0 (0, 3)	NA	NA
Admission to discharge	17 (13, 22)	17 (13, 22.5)	16.5 (14, 23.25)	0.636
Viral co-infections	5/35 (14.29%)	4/21 (19.05%)	1/14 (7.14%)	0.619
Bacterial co-infections	5/35 (14.29%)	3/21 (14.29%)	2/14 (14.29%)	0.531
Complications				
Pneumonia	32/35 (91.43%)	18/21(85.71%)	14/14 (100%)	0.139
ARDS	0/35 (0%)	0/21 (0%)	0/14 (0%)	1.000
Respiratory failure	0/35 (0%)	0/21 (0%)	0/14 (0%)	1.000
Hepatic insufficiency	5/35 (14.29%)	2/21 (9.52%)	3/14 (21.43%)	0.324
Renal insufficiency	0/35 (0%)	0/21 (0%)	0/14 (0%)	1.000

NA: Not applicable.

Characteristics	SARS-CoV-2 childhood cases			P value
	Total (N = 35)	Symptomatic (N = 21)	Asymptomatic (N = 14)	
Cardiac failure	0/35 (0%)	0/21 (0%)	0/14 (0%)	1.000
Shock	0/35 (0%)	0/21 (0%)	0/14 (0%)	1.000
NA: Not applicable.				

Studies based on the adult COVID-19 patients have showed that viral shedding of SARS-CoV-2 in the respiratory and fecal samples appeared as early as the first two days from illness onset (d.a.o), peaked during 3−5 d.a.o, decreased during diseases progression, and persisted up to 37 d.a.o [12, 13]. Similar with adult patients, viral RNAs could be detected in both the respiratory and fecal samples from children patients, and lasted up to 33 and 41 d.a.o, respectively (Fig. 2). Unlike adult patients, viral loads in respiratory samples from children seemed to peaked in the very early stage of disease, usually the first d.a.o, and maintained a middle level during 2−7 d.a.o, then gradually decreased to negative. This observation indicated that viral shedding in children patients might also peak on or before illness onset [13]. Of note, a total of 17 patients (48.57%) were tested positive in the fecal samples, despite that only one patient experienced gastrointestinal symptom. Meanwhile, SARS-CoV-2 shedding in stool is much longer and stable than that in respiratory specimens in children COVID-19 patients (Fig. 2). These results highlighted the importance of detecting viral RNAs in fecal samples of children COVID-19 patients. Recently, live virus was successfully isolated from stool [26], and also we isolated the live virus from the rectal swab of a children patient (data not shown), indicating live virus existed in fecal samples. The longer duration of viral shedding in the fecal samples than the respiratory tract (41 vs 33) and the expression of angiotensin-converting enzyme-2 (ACE2) in intestine enterocytes [27, 28], also highlighted the possibility of SARS-CoV-2 infection and replication in the gastrointestinal tract.

Disease severity varied a lot following SARS-CoV-2 infection in adults, from severe ARDS to asymptomatic infections [4−6]. Studies have found that most of the children patients infected with influenza viruses including H5N1, H7N9, and H5N6 avian influenza viruses (AIVs), as well as coronaviruses like SARS-CoV and MERS-CoV showed asymptomatic and mild symptoms [29−34]. In our study, all the included children cases were moderate and mild cases according to the China National Health Commission Guidelines for Diagnosis and Treatment of SARS-CoV-2 infection. Studies have suggested that lymphopenia may associate with disease severity of COVID-19, as 80% of critically ill adult COVID-19 patients experienced lymphopenia, far higher than the adult patients with mild COVID-19 (25%) [5, 6, 35, 36]. Notably, in our study and some other previous report, only a very small portion of children patients experienced lymphopenia [7], which might result from the relatively immature immune system and different immune response compared with adults [37, 38]. This was different from children patients of SARS-CoV infection, which was reported to induce a high portion of lymphopenia up to 46% [34, 37], indicating a different immune response for the two viruses [37]. Another notification is that CD4

and CD8 were normal in children COVID-19 patients (Table 2). However, our previous study has shown that CD4 and CD8 counts were significantly lower in severe and critically ill COVID-19 patients [18], which indicated that the normal CD4 and CD8 counts might also contribute to the mild COVID-19 in children, as CD4 and CD8 cells has been proved to play vital roles in immune responses, including immune regulation, cytokines secretion, virus-specific antibody production and cytolytic activities against target cells [39].

Previous studies have shown that cytokine storm was highly correlated with disease severity and progression [17, 18], and secretion of pro-inflammatory and anti-inflammatory cytokines were found different among different ages of people [38]. So the expression profile of cytokines in children COVID-19 patients upon admission was also analyzed in this study. Similar with adult patients [16–18], elevated concentrations of both pro- and anti-inflammatory cytokines were observed, despite that adult healthy control was used, indicating that cytokine storm also occurred in children following SARS-CoV-2 infection. However, the number of elevated cytokines was far smaller than the adult patients [17, 18]. Furthermore, cytokines which were shown to be highly correlated with disease severity and progression were significantly lower than adult patients, including IP-10, MCP-3, HGF, MIP-1α, and IL-1ra [17, 18]. These results indicated that much weaker cytokine storm happened in children patients, which might also associate with the milder disease in children.

Conclusions

COVID-19 in children was mild, and asymptomatic infection was common. Immune responses were relatively normal in children COVID-19 patients. Cytokine storm also occurred in children COVID-19 patients, while much weaker than adult patients. Positive rate of viral RNAs in fecal samples was high, and profile of viral shedding were different between respiratory and gastrointestinal tract. To our knowledge, this is the first report on the profile of viral shedding and cytokine expression in children COVID-19 patients, which might provide important information for the understanding of the pathogenesis of SARS-CoV-2 in children.

Abbreviations

Coronavirus Disease 2019, COVID-19; severe acute respiratory syndrome coronavirus 2, SARS-CoV-2; acute respiratory distress syndrome, ARDS; Quantitative reverse-transcriptase polymerase chain reaction, qRT-PCR; China Food and Drug Administration, CDFA; lactate dehydrogenase, LDH; aspartate amino transferase, AST; C-reactive protein, CRP; IL: interleukin; HGF: Hepatocyte growth factor; MCP-3: Monocyte chemotactic protein-3; MIG: Monokine induced gamma interferon; M-CSF: Macrophage colony stimulating factor; G-CSF: Granulocyte colony-stimulating factor; MIP-1α: Macrophage inflammatory protein 1 alpha; CTACK: Cutaneous T-cell-attracting chemokine; IP-10 Interferon gamma induced protein 10;

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Ethics Committees of Shenzhen Third People's Hospital. Verbal informed consents were obtained from all patients or patients family members of COVID-19 patients due to the special circumstances that pens and papers were not allowed to be brought into containment facilities.

Consent to publish

All the authors have read and approved the final version of the manuscript.

Availability of data and material

The datasets supporting the conclusions of this article are included within the article.

Competing interests

The authors declare no conflicts of interest.

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Authors' contributions

YY, HZ, LP and JW has equal contribution. JY, ZZ, LL and YY contributed to the study design. HZ, LP, YW, CC, YL, MC, KF, SF, XW and YL contributed to the collection of clinical specimens and data. JW, HZ, MZ, BP, HL and LX performed the quantitative RT-PCR. YY, HZ and JW contributed to the data analysis. YY wrote the manuscript. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

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Figures
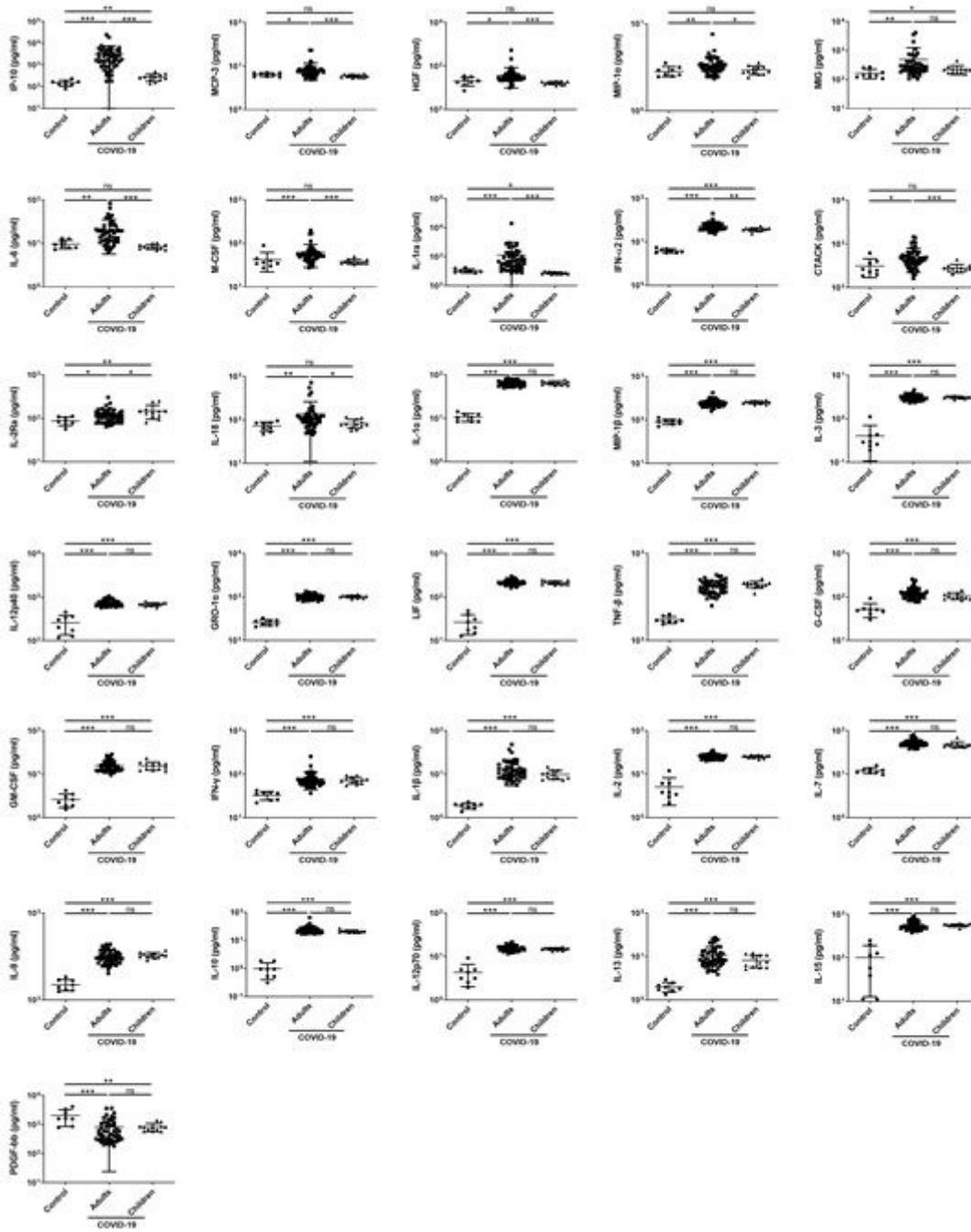
Figure 1

Comparison of serum cytokine concentrations among the children, adult COVID-19 patients and the healthy volunteers. Samples from children (N=11) and adult (N=50) COVID-19 cases were collected at the earliest possible time-point after hospitalization and measured the concentrations of 48 cytokines, and 8 adult healthy subjects were included as control. Values were graphed on a logarithmic scale and presented in units of pg/mL. P values between 0.01-0.05, 0.001-0.01 and 0.0001-0.001 were considered statistically significant (*), very significant (**) and extremely significant (***), respectively, whereas ns represents not significant.
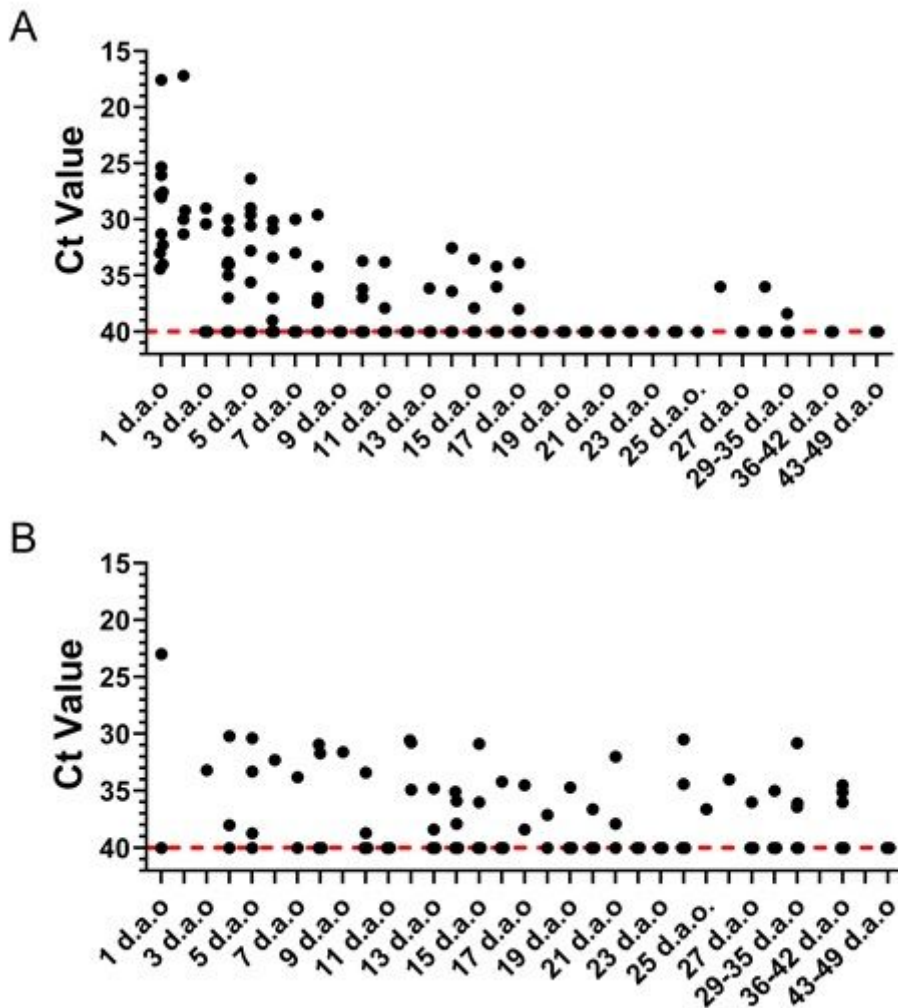
Figure 2

Viral shedding of children COVID-19 patients. Respiratory and fecal samples were collected upon admission and thereafter, and viral RNAs were detected. The obtained Ct values were analyzed in combination with disease progression. The admission date for the asymptomatic patients is defined as the first day after illness onset in this analysis.