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Detectable SARS-CoV-2 Viral RNA in Feces of Three Children during Recovery Period of COVID-19 Pneumonia

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Abstract: Coronavirus Disease 2019 (COVID-19) is a newly emerging infectious disease caused by a novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). After its first occurrence in Wuhan of China from December 2019, COVID-19 rapidly spread around the world. According to the World Health Organization (WHO) statement on March 13, 2020, there had been over 132,500 confirmed cases globally. Nevertheless, the case reports of children are rare, which result in the lack of evidence for preventing and controlling of children's infection. Here, we report 3 cases of SARS-CoV-2 infected children diagnosed from February 3 to February 17, 2020 in Tianjin, China. All of these three cases experienced mild illness and recovered soon after treatment, with the nucleic acid of throat swab turning negative within 14, 11, 7 days after diagnosis respectively. However, after been discharged, all the three cases were tested SARS-CoV-2 positive in the stool samples within 10 days, in spite of their remained negative nucleic acid in throat swab specimens. Therefore, it is necessary to be aware of the possibility of fecal-oral transmission of SARS-CoV-2 infection, especially for children cases.

Keyword: COVID-19; SARS-CoV-2; Children; Clinical characteristics

1. Introduction

COVID-19 caused by a novel coronavirus SARS-CoV-2 infection has rapidly spread worldwide since its onset in Wuhan throughout December 2019 [1-3]. There was a total of 132,536 confirmed cases in 123 countries, areas or territories worldwide, and 4,974 deaths till March13, 2020 [4]. The droplet spread or direct contact with fomites are considered to be the major routes of SARS-CoV-2 transmission [3], while the fecal shedding is also considered to be a minor driver of transmission as viable virus has been identified in a limited number of case reports [5-6]. Most patients infected with SARS-

CoV-2 were mild types and could be cured. About 80% of laboratory-confirmed cases were mild and moderate cases in adult, including those with or without pneumonia, 13.8% of patients were severe illness, and 6.1% of patients were critical illness [7]. Children infected with SARS-CoV-2 were mostly mild cases and few severe cases have been reported [8]. Given that children are in a special immune state, we need to be alert to the occurrence of severe cases. In this study, we collected 3 children cases diagnosed with COVID-19 from February 3 to February 17, 2020 in Tianjin. The clinical characteristics of these children were analyzed and reported in order to provide a reference for the diagnosis and treatment of children during the epidemic of COVID-19.

2. Materials and methods

2.1 Study subjects Children diagnosed with COVID-19 in Tianjin, China, were recruited. The diagnostic criteria for COVID-19 refers to the "New Coronavirus Infected Pneumonia Diagnosis and Treatment Program (Fifth Edition)" [9] issued by the National Health and Health Commission.

2.2 Research method Basic information of children who were diagnosed with COVID-19: clinical characteristics, laboratory tests, chest imaging, etiological examination, treatment and outcome, etc. were collected. The throat swab and fecal specimens were sent to Centers for Disease Control in Tianjin, China. The real-time reverse transcriptase–polymerase chain reaction (RT-PCR) nucleic acid tests were used to detect SARS-CoV-2 RNA.

2.3 Discharge criteria: This study followed the ethical requirements of biomedical research issued internationally and nationally strictly. All the following criteria [9] had to be met for hospital discharge or discontinuation of quarantine: (1) normal temperature lasting longer than 3 days, (2) resolved respiratory symptoms, (3) substantially improved acute exudative lesions on chest computed tomography (CT) images, and (4) 2 consecutively negative RT-PCR test results separated by at least 1 day.

1.1 3. Results

1.1.1 3.1 Clinical Characteristics of Children with COVID-19

All the 3 cases exhibited familial aggregation and experienced a history of close contact with their adult relatives who were diagnosed with COVID-19. All of them were male, aged 9, 6 and 8 years respectively. Throat swab samples were collected on 1-14 days after onset of disease and the results of SARS-CoV-2 nucleic acid tests were positive. All the patients were classified as common type. Case 1 and 3 had fever, nasal obstruction, runny nose and digestive tract symptoms. Case 2 had mild clinical manifestations included cough, expectoration and wheezing. As case 1 combined with supportive tonsillitis, ceftriaxone anti-infection was used. Case 2 was given ribavirin anti-virus treatment. All 3 cases were given interferon atomization, vitamin C, oral Chinese medicine treatment. After treatment, three patients obtained the negative throat swab nucleic acid in 14, 11, 7 days, and were discharged from hospital with 2 times of negative results in 16, 13, 9 days respectively. Follow up of isolation point after discharge: no positive result was found in either of the two times of throat swab nucleic acid tests, but the stool SARS-CoV-2 nucleic acid tests were positive after 10 days. The three patients were re-admitted to the designated hospital. Neither clinical symptoms nor pathological changes in lung imaging were found in any cases. During the monitoring process, three cases appeared SARS-CoV-2 negative of stool specimens in 4, 5 and 10 days respectively (Table 1).

1.1.2 3.2 Laboratory Results of Children with COVID-19

Among the 3 children, case 1 was complicated with purulent tonsillitis, with the increase of leukocyte and C-reactive protein (CRP) with 64.7mg/L, and these indexes reduced to normal level after anti-infective treatment. Case 2 and case 3 showed normal range of white blood cell (WBC), CRP and lymphocyte count. The hemoglobin and platelets were normal in 3 children. There was no obvious abnormality of troponin, blood gas analysis, electrolyte, liver and kidney function or myocardial enzyme in 3 children with COVID-19. Serum procalcitonin (PCT), blood lactate dehydrogenase (LDH), and IL-6 were normal in 3 patients. The D-dimer was normal in all three children, but FIB in cases 2 and 3 were lower than normal. No bacterial growth was observed in the blood culture of

3 children, and mycoplasma pneumoniae, chlamydia, respiratory syncytial virus and adenovirus were negative. Immunological examination (IgG, IgM, IgA, C3, C4) were all in the normal range, and lymphocyte subsets (T cells, B cells, NK cells) were basically normal. Urine and stool are normal. No abnormality was found either in electrocardiogram (ECG) and echocardiography, or in liver, gallbladder, and kidney ultrasound (Table 2).

1.1.3 3.3 Imaging Characteristics of Children with COVID-19:

In this study, 3 cases of children with lung computed tomography (CT) were found positive, and 2 of them mainly showed ground-glass opacity (GGO), and 1 of them showed new cord shadow, which were located in the lower lung, outer band, near the pleura, and the scope was small and more limited. Review of CT after treatment showed that the lesions were absorbed to varying degrees (3-5 days). Specific imaging manifestations and dynamic changes are as follows:

Case 1: Admission lung CT: There was no lesion in anterior medial basal segment of the left lower lobe (Fig.1a). Reexamination of the lung CT in 4 days after admission: new thin strips of anterior medial basal segment of the left lower lobe (Fig.1b). Review of lung CT in 7days after admission: left lower lobe lesions improved and absorbed (Fig.1c). Review of lung CT in 12 days after admission: left lower lobe anterior interior basal lesions were significantly smaller than before (Fig.1d).

Case 2: Admission lung CT: light GGO in the anterior segment of the outer basal segment of the right lower lobe is located under the pleura (Fig.1e). Ring slightly high-density shadow can be seen in the lesions of the right lower lobe. Lung CT was rechecked 5 days after admission: the GGO of the two lungs was slightly absorbed and faded (Fig.1f).

Case 3: Admission lung CT: small piece of GGO under the pleura of the posterior basal segment of the left lower lobe, with uneven density and fuzzy edge (Fig.1g). Reexamination of lung CT 4 days after admission: the range of lesions in the left lower lung was reduced and the density became weak (Fig.1h).

1.2 4. Discussion

Since December 2019, there were multiple cases of patients with COVID-19 pneumonia found in Wuhan city of Hubei province. As the SARS-CoV-2 spreading around the whole world, COVID-19 has become a global pandemic disease [10-12]. Pediatric case reports were gradually emerging [13-14], even newborn COVID-19 case has been reported [15-16]. Childhood infections were mainly caused by family clustering outbreaks and imported cases [8,17], daily prevention at home was the main method to prevent viral spreading among children. 3 cases of children in this study contacted with close relatives who were diagnosed with COVID-19. The 3 children in this study were classified as a common type of COVID-19, with no severe symptom. After infection, children's first symptoms were not typical, as most of them only got fever and cough [6,8], and some suffer from gastrointestinal symptoms [15]. Except for case 1 with supportive tonsillitis, and obvious fever symptoms, no other serious symptoms were found in all of the 3 cases. Cases 1 and 3 had mild digestive tract symptoms, while case 2 and case 3 showed normal range of CRP, WBC and lymphocyte count, which consistent with other studies [8,17]. The lung CT showed ground glass density shadow in 2 cases, which was typical manifestation of COVID-19 pneumonia [18-20]. As no specific anti-viral drug available for SARS-CoV-2 infection to date, 3 patients were all treated with interferon nebulization, vitamin C, and Chinese medicine orally and had good results.

In the follow-up after discharge, all patients with COVID-19 had positive stool RT-PCR test results after 10 days, accompanied by their member of family. There were no clinical symptoms or imaging discovered in the patients. No positive findings were found by two times of throat swab RT-PCR tests. Considering that children hardly cooperate in taking samples, further study is still necessary to assess whether two negative throat swab tests can be feasible indicator to judge children's discharge and isolation. It has been reported that SARS-CoV-2 nucleic acid was detectable in stool of adults and anal swabs of children [6,21], which suggested that live virus exist in the feces discharged by the patients. In our study, we found that the stool nucleic acid was still positive after 10 days of recovery, and compared with anal swab specimens, stool specimens are more

representative, suggesting the long existence of viruses in feces and on the surface of objects.

Recovered patients might be possible carriers for the viruses, which force us to reevaluate the current criteria of hospital discharge or discontinuation of quarantine and continued patient management. The study was limited to a small number of patients, longitudinal studies on a larger cohort would help to understand the prognosis of the disease.

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Conflict of interest

The authors have no Conflicts of interest to declare.

Authors' contributions

All authors contributed to the intellectual content of this manuscript and approved the final manuscript as submitted. Tongqiang Zhang, Xiaojian Cui and Xue Zhao drafted the initial manuscript. Jinhu Wang, Jiafeng Zheng, Wei Guo, Chunquan Cai and Guifen Zheng cared the patient and collected the clinical samples or data. Sijia He and Yongsheng Xu revised the article critically for important intellectual content.

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Data sharing statement

The data used in this report are available from the corresponding author on reasonable request.

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Figure 1. Imaging Characteristics of Children with COVID-19. Admission lung CT of case 1 (a, b, c and d), case 2 (e and f), case 3 (g and h).

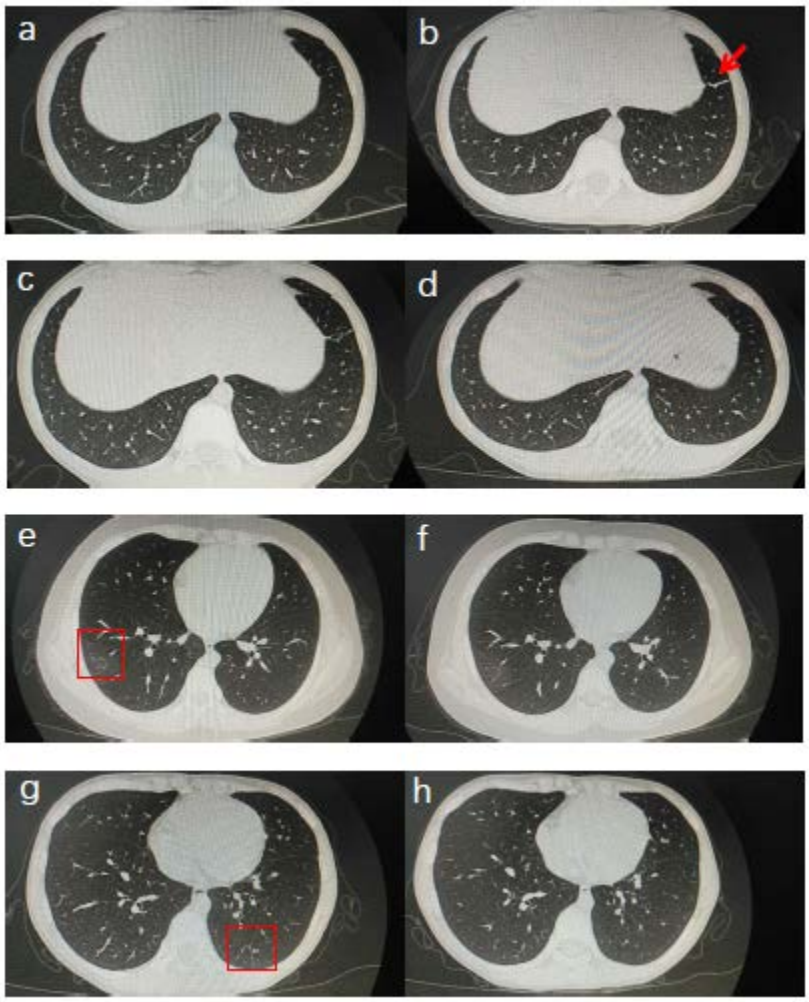

Table 1 General information of children with COVID-19

Basic Features	Case1	Case2	Case3
Age (yr)	9	6	8
Gender	male	male	male
Time from onset of symptoms to diagnosis (d)	1	14	7
Clustered cases within 14 days	Yes	Yes	Yes
Contact with fever patients in Wuhan within 14 days	Yes	No	No
Fever	Yes	No	Yes
Sore throat	Yes	No	No
Nasal congestion, runny nose	Yes	No	Yes
Fatigue	No	No	No
Cough	No	Yes	No
Gastrointestinal symptoms	Nausea, gastric appetite	No	Gastric appetite

Restless	No	No	No
Headache, muscle pain	Yes	No	No
Treatment	Interferon, Chinese medicine, vitamin C	Interferon, Chinese medicine, vitamin C	Interferon, Chinese medicine, vitamin C
Hormone use	No	No	No
Antibiotic application	Yes	No	No
Time from diagnosis to throat swab SARS-CoV-2 nucleic acid negative (d)	14	11	7
Length of hospital stay (d)	16	13	9
Time from discharge to stool SARS-CoV-2 nucleic acid positive (d)	13	11	10
Time form re-admission to stool SARS-CoV-2 nucleic acid negative (d)			

Table 2 Laboratory test results of children with COVID-19

Laboratory Indicators	Case 1	Case 2	Case 3	Normal Range
HB (g/L)	114	134	130	120-160
WBC count ($\times^9$/L)	10.65	7.99	6.42	4-10
N%	58.5	72.3	34.2	50-75
Lymphocyte count ($\times^9$/L)	1.46	1.43	3.62	0.8-4
L%	13.7	17.9	56.4	20-40
CRP (mg/L)	64.7	6.28	0.2	0-10
PCT (ng/ml)	0.102	0.04	0.04	0-0.5
ALT (U/L)	26	24	15	21-72
AST (U/L)	30	23	25	17-59
CK (U/L)	39	45	37	55-170
CKMB (ug/L)	7	11	10	0-25
cTnI (ng/ml)	0.012	0.012	0.012	0-0.12

IL-6 (pg/ml)	25.7	2.7	1.5	0-10
LDH (U/L)	467	423	495	313-618
D-dimer (mg/L)	0.112	0.34	0.28	0-0.55
FIB (g/L)	2.9	1.78	1.85	2.0-4.0

Note: HB: hemoglobin, WBC: white blood cell, N%: neutrophil ratio, L%: lymphocyte ratio, CRP: C-reactive protein, PCT: procalcitonin, ALT: alanine aminotransferase, AST: aspartate aminotransferase, CK: creatine Kinase, CKMB: Creatine kinase Mb isoenzyme, cTnI: marker of myocardial injury, IL-6: interleukin-6, LDH: lactate dehydrogenase, FIB: fibrinogen.