European Review for Medical and Pharmacological Sciences 2020; 24: 5772-5777

No infectious risk of COVID-19 patients with long-term fecal 2019-nCoV nucleic acid positive

Q.-X. WANG[1], K.-C. HUANG[1], L. QI[2], X.-H. ZENG[3], S.-L. ZHENG[4]

[1]Department of Infection Management, Affiliated Hospital of Weifang Medical University, Weifang, Shandong, P.R. China
[2]Department of Infectious Disease, Affiliated Hospital of Weifang Medical University, Weifang, Shandong, P.R. China.
[3]Department of Lung Center, Weifang Yangguang Ronghe Hospital, Weifang, Shandong, P.R. China
[4]Department of General Medicine, Affiliated Hospital of Weifang Medical University, Weifang, Shandong, P.R. China

Abstract. – OBJECTIVE: It has recently been reported that some COVID-19 patients have long-term positive fecal nucleic acid after discharging from the hospital with negative nucleic acid in the respiratory tract, but it is unclear whether COVID-19 patients with positive long-term fecal nucleic acid tests have the risk of self-infection.

PATIENTS AND METHODS: From January 25, 2020 to March 9, 2020, 5 COVID-19 patients with negative respiratory tract nucleic acid and positive fecal nucleic acid were observed and studied to explore whether these patients can re-infect themselves. Five patients with COVID-19 accompanied by diarrhea as the main gastrointestinal symptoms were carefully observed through clinical symptoms, imaging and other auxiliary examinations. The RT-PCR technology was used to continuously detect fecal and respiratory viral nucleic acids. The IgM antibody was detected on the 7th day of admission and IgM/IgG at the time of discharge.

RESULTS: All 5 patients had symptoms of fever and diarrhea upon admission. The fecal nucleic acid was positive, as well as the throat swab was positive. All COVID-19 patients had positive IgM antibodies on the 7th day of admission and positive IgM and IgG at the time of discharge, and there were no abnormalities in the gastrointestinal examination on discharge. All 5 fecal nucleic acid tests were positive at the time of discharge. After continuous dynamic follow-up for 3-15 days, no clinical symptoms recurred, and the last nucleic acid test was negative.

CONCLUSIONS: There is no risk of self-infection for COVID-19 patients with long-term 2019-nCoV nucleic acid positive in feces.

Key Words:
SARS-CoV-2, COVID-19, Self-infection, Nucleic acid test, Feces.

Introduction

In December 2019, there was an outbreak of pneumonia characterized by fever and expectoration in Wuhan, Hubei Province. Most of the cases were related to the South China Seafood Market in Wuhan[1]. Infected patients developed fever and a cough rapidly and most severe cases developed difficulty breathing one week after the onset of the disease, while the mild cases only showed low fever and slight fatigue, and some patients developed diarrhea[2]. On January 7, 2020, the novel coronavirus was identified by the Chinese Center for Disease Control and Prevention (CDC) from patients' throat swab samples, and the disease was latterly named COVID-19 (Coronavirus Disease 2019) by the World Health Organization (WHO)[3].

Since February 1, 2020, some confirmed patients with COVID-19 in Wuhan and Shenzhen were tested positive with fecal 2019-nCoV nucleic acids, and the virus was also detected in the feces of the first confirmed patient with COVID-19 in the United States[4]. In March 2020, it was found that, after the patients' respiratory tract samples were negative for 2019-nCoV, some of their fecal samples were positive for the virus for a long time. Therefore, many health workers are concerned about the risk of fecal-oral transmission of those patients, and doctors are unsure of how to deal with this condition. In the current study, the clinical symptoms and fecal nucleic acid changes were analyzed, and auxiliary examinations were performed among five patients with long-term fecal 2019-nCoV nucleic-acid-positive and diarrhea.

Corresponding Author: Shiliang Zheng, MD; e-mail: wyfyqkyx@163.com

Patients and Methods

Study Participants

Five patients with COVID-19-related diarrhea who were admitted to our hospital in Weifang from January 25, 2020, to March 9, 2020, were enrolled. The participants all meet the diagnostic standards of the "COVID-19 Diagnosis and Treatment Program (Trial Fifth Edition)" issued by the National Health and Health Commission (ref).

The epidemiological data, clinical symptoms, and laboratory and imaging results for those patients were recorded. The 2019-nCoV nucleic-acid test in feces and throat swab were detected by Polymerase Chain Reaction (RT-PCR). Among the patients, there were two males and three females aged on average 42 (35-56) years. Signed informed consent was obtained from all five patients.

Observation Methods

Information on the clinical symptoms of patients with COVID-19, such as fever, cough, and diarrhea, was collected. After admission, lymphocyte counts, and liver function markers of the blood were examined several times according to patients' condition. The levels of 2019-nCoV-Immunoglobulin M (IgM) antibodies were detected by gold-labelled Western blotting on the 7th day of admission and IgM and Immunoglobulin G (IgG) antibodies at the time of discharge.

Nucleic acids of COVID-19 in feces were detected by Real-time fluorescent quantitative RT-PCR. Throat swabs were used to detect nucleic acids repeatedly according to the need for diagnosis after admission. According to changes in the disease, a chest CT examination was performed every 2-4 days to observe the changes in lung during hospitalization, while abdominal ultrasound, abdominal CT, and whole-digestive barium meal fluoroscopy were performed on the day of discharge to assess the gastrointestinal lesions.

Clinical discharge conditions included a normal body temperature for more than 3 days, significant improvement of respiratory symptoms, and significant absorption of pulmonary CT lesions. The patients were discharged after two negative 2019-nCoV nucleic-acid examinations of throat swabs. The nucleic acids were analyzed using RT-PCR from throat swabs many times, in accordance with the clinical discharge conditions. Meanwhile, a fecal nucleic acid test was conducted every 3 days. A dynamic observation of fecal nucleic acids, fever, and diarrhea was performed to analyze the self-infection risk of long-term fecal nucleic-acid-positive-patients with COVID-19 diarrhea.

Throat Swab and Fecal Sample Collection

Samples were collected 2 hours after the patients had eaten. The swabs were used with sterile normal saline (swabs were not placed in the virus storage solution to prevent antibiotics from causing allergies). The patients were asked to gargle with normal saline with their head slightly tilted back and mouth open wide, accompanied by an "ah" sound. With the bilateral pharyngeal tonsils exposed, the swab was placed across the base of the tongue and used to gently rub back and forth across the bilateral pharyngeal tonsils at least three times, then wiped across the posterior pharyngeal wall at least three times. The swab head was placed in a tube containing 2 to 3 ml of virus preservation solution (also isotonic saline solution, tissue culture solution, or phosphate buffer solution), the stick was discarded, the tube was tightly sealed, and the sample was sent out for analysis. The fecal samples were collected in the morning and put into dedicated receptacles for specialized analysis.

Virus Nucleic Acid Testing

The throat swab and fecal samples from all patients were stored in virus collection media and sent to the Laboratory of Weifang Center for Disease Control and Prevention, where the presence of the 2019-nCoV strain was detected by RT-PCR. Ribonucleic Acid (RNA) was extracted from the patient samples and RT-PCR was performed. The virus information in the samples was amplified by PCR. If the signal was positive on PCR, the test was repeated to further verify the test results.

Statistical Analysis

All experiments were repeated in triplicate, and all data were presented as the average of three independent experiments. Data were analyzed with SPSS (version 26.0, IBM Corp., Armonk, NY, USA) and presented as median (range).

Results

Clinical Symptoms

On admission, five patients had a fever; two patients had a fever of >38.5°C, while three had temperatures of 38.0°C, 37.8°C, and 37.5°C, respectively, and the fever lasted for 7 (3-10) days. Interestingly, all five patients had diarrhea lasting for 5 (3-9) days. At the same time, three cases had cough lasting for 7 (3-10) days. As shown in Table I, no patients had nasal congestion, runny nose, sneezing, or difficult breathing.

Auxiliary Examination

Five patients had decreased lymphocyte with a duration of 7 (3-11) days, and liver function was normal in all patients. After admission, two patients were positive once for throat swab nucleic acids, two patients were positive twice, and one patient was positive three times. In all patients, only IgM antibodies were positive on the 7th day of admission and on discharge, but both IgM and IgG at the time of discharge. Regarding chest CT, one patient had no abnormalities and the other four patients had ground-glass opacity or patchy opacity with evident absorption for 9 (7-12) days (Figure 1). There were no bile duct abnormalities on hepatobiliary ultrasound or abdominal CT and no gastrointestinal organic lesions were found on whole-digestive barium-meal fluoroscopy for all five patients.

Follow-Up Results After Discharge of Patients With COVID-19

The first nucleic acid tests in feces on discharge day were positive in all patients. After discharge, fecal nucleic acid tests positive in 5 cases continued for 3-15 days, while all the patients had no clinical symptoms during this period. This result indicated that there is no self-infectious risk of COVID-19 patients with long-term 2019-nCoV nucleic-acid-positive in feces.

Discussion

The current study of five patients with COVID-19-related diarrhea, who were fecal nucleic-acid-positive for a long time, revealed that (1) all the patients with COVID-19-related diarrhea were positive for fecal nucleic acids; (2) the duration of positive nucleic-acids in fecal samples (up to 15 days) was significantly longer than that in throat swab samples. (3) Patients who develop IgM and IgG antibodies remain fecal nucleic acid positive for long periods of time, but do not re-infect themselves.

All five patients with COVID-19-related diarrhea had positive fecal nucleic acids, which were not a result of sepsis spreading to the gastrointestinal tract because all the patients were of ordinary type and their clinical manifestations were not serious. It has been demonstrated that approximately 0.64% of human lung cells express angiotensin converting enzyme 2 (ACE2), and 83% of these cells are type II alveolar epithelial cells (AT2). The type II alveoli that express ACE2 account for approximately 1.4% of all AT2 cells, suggesting that COVID-19 is caused by S-protein interacting with human ACE2 and the virus infects the alveoli of the lower respiratory tract rather than the upper respiratory tract[5]. Zhou et al[6] showed that the S-protein of 2019-nCoV mainly binds to ACE2 on the surface of human cells and that ACE2 is abundant in the epithelial cells of human lung and small intestine.

In this study, five patients with COVID-19 had both pulmonary and gastrointestinal symptoms; therefore, diarrhea may have been caused by a direct attack of 2019-nCoV on the intestines. Specifically, 2019-nCoV may enter the alveoli and small intestines via the throat by respiration and swallowing then attack the corresponding sensitive target cells to cause disease. In current study, one patient showed no lung injury and only significant gastrointestinal symptoms, which imply the differences of ACE2 expression among individuals.

Ling et al[7] have demonstrated that ACE2 expression may be as high as 59.7% in the bile duct cluster, which is a potential route for viral infection that could lead to direct bile duct injury. In addition, researchers have found that ACE2 is highly expressed in human small intestines. Interestingly, the level of ACE2 RNA in lung tissues is very low, small intestinal epithelial cells expressing ACE2 may be susceptible to 2019-nCoV infection, and there is evidence that diarrhea is an indicator of 2019-nCoV infection, suggesting that clinicians should pay more attention to diarrhea patients during COVID-19 outbreaks. Current studies showed that five patients with COVID-19 had normal liver function, no bile duct abnormalities on hepatobiliary ultrasound and abdominal CT, and no gastrointestinal organic lesions on whole-digestive barium meal fluoroscopy. The imaging examinations were conducted at the time of discharge, when gastrointestinal symp-

Table I. Symptom, image, and COVID-19 RNA test results.

Close contact days		1	3	5	7	9	10	11	12	15	18	21	24	27	30	33
Case 1	Fever	√	√	√	√	√	√									
	Diarrhea	√	√	√	√	√										
	Nausea & vomiting	√	√	√	√											
	Cough	√	√	√	√	√	√									
	Lymphocyte count	↓	↓	↓	↓	↓	↓	↓	–							
	CT	■	■		■		■		□							
	IgM, IgG				•					•★						
	COVID-19 RNA Test	◆▲			◆	◆	◇	◇		▲	▲	▲	▲	▲	▲	△
Case 2	Fever	√	√	√	√	√										
	Diarrhea	√	√	√	√											
	Nausea & vomiting	√	√	√												
	Cough	√	√	√	√											
	Lymphocyte count	↓	↓	↓	↓	↓	-									
	CT	■		■	■		□									
	IgM, IgG				•				•★							
	COVID-19 RNA Test	◆▲		◆			◇	◇	▲	▲	▲	▲	▲	△	△	
Case 3	Fever	√	√	√	√											
	Diarrhea	√	√	√												
	Nausea & vomiting	√	√													
	Cough	√	√													
	Lymphocyte count	↓	↓	↓	↓	-										
	CT	■		■		□										
	IgM, IgG				•				•★							
	COVID-19 RNA Test	◇▲	◇	◆	◆		◇	◇	▲	▲	▲	▲	△	△		
Case 4	Fever	√	√													
	Diarrhea	√	√													
	Nausea & vomiting															
	Cough															
	Lymphocyte count	↓	↓	-												
	CT															
	IgM, IgG				•	•★										
	COVID-19 RNA Test	◇▲	◇	◆	◇	◇▲			△	△						
Case 5	Fever	√	√	√												
	Diarrhea	√	√	√												
	Nausea & vomiting															
	Cough															
	Lymphocyte count	↓	↓	↓	↓	-										
	CT	■	■	■	□											
	IgM, IgG				•				•★							
	COVID-19 RNA Test	◇▲	◇	◇	◆		◇	◇	▲	▲	△	△				

Footnote: √Positive of clinical symptoms; ■ Positive of chest CT; □ Negative of chest CT; ◆ Positive of nucleic acid test for throat swab; ◊ Negative of nucleic acid test for throat swab; ▲ Positive of nucleic acid test for feces; Δ Negative of nucleic acid test for feces; ● Positive of IgM test; Δ Positive of IgG test.

toms such as diarrhea have been improved. Thus, it is possible that the imaging failed to show 2019-nCoV invasion of the bile duct, improvement of intestinal lesions, or minor lesions.

As yet, no cases of 2019-nCoV fecal-oral transmission have been reported, indicating that infection through this route while in hospital or self-isolation is unlikely. The five patients en-

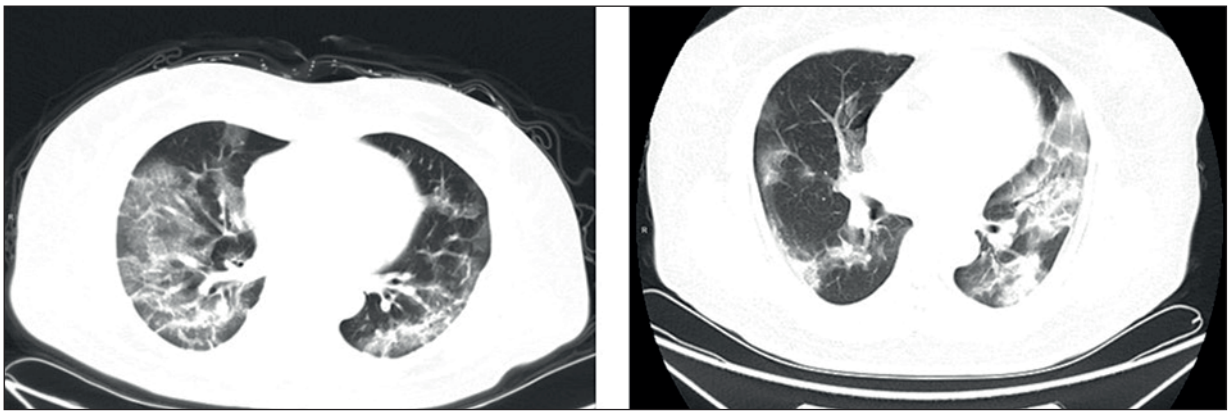

Figure 1. Chest CTs of two patients with COVID-19. Chest CT obtained when diagnosed as COVID-19 showed mass shadows of high density in both lungs. Bright bronchogram is seen in the lung tissue area of the lesion.

rolled in current study were discharged from hospital after throat swab nucleic-acid negative on two consecutive tests; however, they were positive for serum anti-2019-nCoV-IgM on two consecutive occasions and for IgG at discharge. This shows that, even if the patient comes into contact with the virus in his own feces, infection did not occur. Wu et al[8] in bioRxiv, on February 27, 2020, revealed that the prong protein structure of 2019-nCoV can combine with an antibody, which also proves the protective effect of antibody. In addition, no clinical symptoms were found during the period of positive fecal nucleic acid tests after discharge, proving that there was no self-infection in these patients.

According to our results, we recommend that patients with COVID-19-related diarrhea should be monitored for fecal nucleic acids test, but this is not required for those without diarrhea; while patients with COVID-19-related diarrhea who have positive fecal samples should be tested for serum antibodies. Finally, the dynamic observations of patients with long-term fecal 2019-nCoV-positive samples are needed to further study the survivability and infectivity of the virus in feces.

Conclusions

Briefly, there is no risk of self-infection through fecal-oral transmission for the COVID-19 patients with long-term positive nucleic acid in feces.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Ethical Statement

All analyses were based on previous published studies; thus, no ethical approval and patient consent are required.

References

1) Chan JF, Yuan S, Kok KH, To KK, Chu H, Yang J, Xing F, Liu J, Yip CC, Poon RW, Tsoi HW, Lo SK, Chan KH, Poon VK, Chan WM, Ip JD, Cai JP, Cheng VC, Chen H, Hui CK, Yuen KY. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. Lancet 2020; 395: 514-523.
2) Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y, Ren R, Leung KSM, Lau EHY, Wong JY, Xing X, Xiang N, Wu Y, Li C, Chen Q, Li D, Liu T, Zhao J, Liu M, Tu W, Chen C, Jin L, Yang R, Wang Q, Zhou S, Wang R, Liu H, Luo Y, Liu Y, Shao G, Li H, Tao Z, Yang Y, Deng Z, Liu B, Ma Z, Zhang Y, Shi G, Lam TTY, Wu JT, Gao GF, Cowling BJ, Yang B, Leung GM, Feng Z. Early transmission dynamics in Wuhan, China, of Novel Coronavirus-infected pneumonia. N Engl J Med 2020; 382: 1199-1207.
3) Zhou Z, Zhao N, Shu Y, Han S, Chen B, Shu X. Effect of gastrointestinal symptoms on patients infected with COVID-19. Gastroenterology 2020 Mar 18. pii: S0016-5085(20)30362-0. doi: 10.1053/j.gastro.2020.03.020. [Epub ahead of print]
4) Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, Qiu Y, Wang J, Liu Y, Wei Y, Xia J, Yu T, Zhang X, Zhang L. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet 2020; 395: 507-513.
5) Holshue ML, DeBolt C, Lindquist S, Lofy KH, Wiesman J, Bruce H, Spitters C, Ericson K, Wilkerson S, Tural A, Diaz G, Cohn A, Fox L, Patel A, Gerber SI, Kim L, Tong S, Lu X, Lindstrom S, Pallansch MA, Wel-

don WC, Biggs HM, Uyeki TM, Pillai SK, Washington State -nCo VCIT. First case of 2019 novel Coronavirus in the United States. N Engl J Med 2020; 382: 929-936.

6) Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, Si HR, Zhu Y, Li B, Huang CL, Chen HD, Chen J, Luo Y, Guo H, Jiang RD, Liu MQ, Chen Y, Shen XR, Wang X, Zheng XS, Zhao K, Chen QJ, Deng F, Liu LL, Yan B, Zhan FX, Wang YY, Xiao GF, Shi ZL. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 2020; 579: 270-273.

7) Liang W, Feng Z, Rao S, Xiao C, Xue X, Lin Z, Zhang Q, Qi W. Diarrhoea may be underestimated: a missing link in 2019 novel coronavirus. Gut 2020 Feb 26. pii: gutjnl-2020-320832. doi: 10.1136/gutjnl-2020-320832. [Epub ahead of print].

8) Wu Y, Guo C, Tang L, Hong Z, Zhou J, Dong X, Yin H, Xiao Q, Tang Y, Qu X, Kuang L, Fang X, Mishra N, Lu J, Shan H, Jiang G, Huang X. Prolonged presence of SARS-CoV-2 viral RNA in faecal samples. Lancet Gastroenterol Hepatol 2020; 5: 434-435.