Detection of SARS-CoV-2 in Different Specimens of Inpatients, Covert Infectors and Discharged Persons

Xiaorong Yang (yangyangxr@163.com)
Sichuan Center for Disease Control and Prevention https://orcid.org/0000-0002-5676-5761

Linzi Zeng
Sichuan Center for Disease Control and Prevention

Yulan Huang
Sichuan center for disease control and prevention

Ping Ye
Ganzi center for disease control and prevention

Zicheng Tian
Ganzi Center for Disease Control and Prevention

Jiushun Zhou
Sichuan Center for Disease Control and Prevention

Wei Li
Ganzi center for disease control and prevention

Short Report

Keywords: novel cronavirus, various biological samples, mild patients, asymptomatic infection

Posted Date: June 4th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-29279/v1

Abstract

With emergence of pandemic COVID-19, the detection of virus in clinical samples is important. This study compared different clinical samples detectd from inpatient, covert infector and discharged persons, Overall, Some new possible transmission of the virus by patient,covert or discharged person were unknown..

Introduction

An epidemic of respiratory disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) began in China on December 21, 2019, and spread quickly to other countries.[1] The two commonly used nucleic acid detection technologies for SARS-CoV-2 are real-time reverse transcriptase–polymerase chain reaction (rRT-PCR) and high-throughput sequencing.[2]. However, the application of high-throughput sequencing technology in clinical diagnosis is limited because its equipment dependency and high cost. Therefore rRT-PCR is the most common and effective method for detecting SARS-CoV-2 in specimens. Real-time reverse transcriptase–polymerase chain reaction of espiratory tract specimens in the acute phase (including upper or lower respiratory tract specimens)has been used to confirm the clinical diagnosis. The nucleic acid test of respiratory tract specimens was negative twice in a row(the sampling time should be at least 24 hours apart) has been used for discharge and release of isolation [3]. The virus had be detected in Blood, sputum, feces, urine and nasal samples in patients and covert infectors, [4]. However, it’s to be confirmed whether have the difference between COVID-19 patients and covert infectors. Whether the virus can be detected in discharged persons according to the standard and the virus carrying period of the COVID-19 patient and the covert infector need to be testified as well.Some new possible transmission of the virus by discharged persons is unknown.

Methods

We collected pharyngeal swab, saliva, urine, feces and hand swab specimens of COVID-19 inpatients and covert infectors diagnosed based on symptoms and radiology and confirmed by SARS-CoV-2 nucleic acid detection in hospitals, we collected urine, pharyngeal swab and feces specimens of discharged patients and covert infections in isolation points. Meanwhile, the discharge persons with positive feces have been monitored continuously until the feces are tested negatively. This study has been approved by the ethics commissions of the participating hospitals, with a waiver of informed consent.

Inpatients and covert infectors with specimens collected on day 4 to 20 after entering the hospital based on clinical indications and nucleic acid test result from 2 hospitals, discharged persons with specimens collected on day 1to 35 after entering the isolation spot based on the novel coronavirus infection diagnosis and treatment plan from 3 isolation spots[3], in the Daofu county, Ganzi Tibetan Autonomous Prefecture, Sichuan province, China, from February 22 through March 27, 2020, were included. RNA was extracted from specimens by using the Natch S system (Sansure Biotech), and determined by rRT-PCR targeting the open reading frame*1ab* (ORF 1ab) gene and N gene of SARS-CoV-2 using SARS-CoV-2 nucleic acid detection kit(Sansure Biotech). A cycle threshold value less than 40 was interpreted as positive for target. The specimen was determined to be positive for SARS-CoV-2 RNA when both targets were positive .

Results

A total of 599 specimens were collected in this study, of which 238 specimens collected from 46 cases (including 33 patients with COVID-19 and 13 covert infectors) in the hospital, 33 patients with COVID-19 who were an average age of 48 years (range,7 to 77 years) and 58% male. Most of the patients presented with fever, dry cough, and fatigue; none of patients had severe illness. 13 covert infectors who were an average age of 36 years (range, 11 to 52 years) and 77% male. Of which 233 specimens were collected from 93 discharged persons (including 73 discharged patients and 20 discharged covert infectors) in the isolation points, 93 discharged persons who were a mean age of 39 years (range, 3 to 77 years) and 57% male. 128 specimens were collected from 18 discharged persons whose fecal were positive.

The feces specimens showed the highest positive rate(15 of 31, 48.4%), followed by pharyngeal swabs(21 of 46, 45.6%), urine (9 of 53,17%), hand swabs(7 of 38,18.4%) and finally saliva (7 of 70,10%) in the specimens collected in the hospital. The pharyngeal swabs specimens showed the highest positive rate(18 of 35,51.4%), followed by feces specimens(11 of 24, 45.8%), urine (9 of 49,18.4%), hand swabs(7 of 34, 20.6%) and saliva (7 of 57, 12.3%) in 33 inpatients specimens. The feces specimens showed the highest positive rate(4 of 7, 57.1%), followed by pharyngeal swabs(3 of 11, 27.3%) in 13 covert infectors specimens who stay in hospital and in which none of urine, hand swabs and saliva tested positive.Only the feces specimens detected positive (18 of 93, 19.4%), including 15 discharged patients and 3 discharged covert infectors in the specimens collected in the isolated spots. None of 93 pharyngeal swabs and 47 urine tested positive. (table)

Eighteen discharged persons had positive feces specimens for a mean of 21 days (range:from 9 to 33 days)continuously after their respiratory samples became negative. They have been tested positive for SARS-CoV-2 RNA in their clinic specimens for a mean of 37 days (range:from 29 to 48 days) after first symptom onset or the first clinic specimens tested positive for SARS-CoV-2 RNA. Among them, 3 discharged covert infectors had positive feces specimens for a mean of 20 days (range: from 16 to 22 days) continuously after they respiratory samples became negative. The clinic specimens were tested positive for SARS-CoV-2 RNA for a mean of 32 days (range: from 29 to 35 days) after first clinic specimens tested positive for SARS-CoV-2 RNA (figure).

Six patients' specimens were collected three times in hospital. 12 patients' specimens were collected two times. All persons were collected 2–5 different specimens simultaneously each time. Viral RNA was detected simultaneously in the saliva, feces, pharyngeal swabs and hand swab specimens from 2 patients. Viral RNA was detected simultaneously in feces and urine specimens from 5 patients. Viral RNA was also detected simultaneously in respiratory tract specimens and in feces from 9 patients.

Conclusion

In this study, SARS-CoV-2 RNA has been detected from different specimens of patients,covert infectors and discharged persons. Viral RNA could be detected simultaneously in several clinic specimens. They have been tested positive for SARS-CoV-2 RNA in their clinic specimens for a mean of 37 days (range:from 29 to

48 days) after first symptom onset or the first clinic specimens tested positive for SARS-CoV-2 RNA.When the patient met the discharge standard, the respiratory tract samples were negative for 24 hours in a row, and the viral RNA was still detected in the feces. The dischargeed person had positive feces specimens for a mean of 21 days continuously after their respiratory samples became negative.

Discussion

The SARS-CoV-2 RNA Viral RNA could be detected simultaneously in several clinic specimens which suggested that different specimens can be collected simultaneously to improve the sensitivity and reduce false-negative test results. There were studies reported the live SARS-CoV-2 virus had been detected in feces and urine specimens[5], implying that SARS-CoV-2 may be transmitted by respiratory and extrarespiratory routes. This study showed that when saliva was positive, pharyngeal swabs was positive too, otherwise it did not hold. Viral RNA were detected in hand swabs and saliva specimens, suggesting that serving dishes individually, no shaking hands and washing hands more frequently are helpful to stop the transmission of the virus.

It has been reported that the virus carried by covert infectors were still infectious[6],In this study, The mean continuous positive detection period was 31 days from covert infectors. Therefore, suggesting to track and supervise covert infectors. When the patient met the discharge standard, the respiratory tract samples were negative for 24 hours in a row, and the viral RNA was still detected in the feces. In this study, we found that 19% (18 / 93) of the respiratory tract samples were negative, and the feces continued to tested viral RNA positive for an average of 21 days. implying that the virus is actively replicating in the Patient's gastrointestinal tract and that faecal–oral transmission could occur after viral clearance in the respiratory tract,Therefore it is recommended that the discharge person should be under observation continuously until the feces become negative.

Declarations

Ethical Approval and Consent to participate: This study has been approved by the ethics commissions of the participating hospitals, with a waiver of informed consent.

Consent for publication:YES

- **Availability of data and materials:**NO

- **Competing interests:** None reported.

- **Funding:** This work was supported by Sichuan science and Technology Program(2020YFS0015).

- Authors' contributions:

Yang ,Zeng and Huang had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Yang ,Zeng and Huang contributed equally.

Concept and design: Yang.

Sample collection, testing and data analysis or interpretation of data: Yang,Zeng,Huang, Ye, Tian.

Drafting of the manuscript: Yang.

Obtained funding: Yang.

Administrative, technical, or material support: Zhou, Li.

Supervision: Zhou, Li.

Acknowledgements: Not applicable.

- **Authors' information:**

Jiushun Zhou: E-mail:zzzzjs@hotmail.com

References

1.W Tan, X.Zhao , X Ma, *et al*. Notes from the field: a novel coronavirus genome identified in a cluster of pneumonia cases—Wuhan, China 2019-2020. *China CDC Weekly*. 2020;2(4):61-62.

2.P. Zhou, X. L.Yang, X. G. Wang, *et al.*, A pneumonia outbreak associated with a new coronavirus of probable bat origin, Nature (2020). https://doi.org/10.1038/s41586-020-2012-7

3.General Office of the National Health Commission, Office of State Administration of TraditionalChinese Medicine. Notice on the novel coronavirus infection diagnosis and treatment plan (trial version seventh) [EB/OL]. (2020-03-03).

4.GUANGZHOU DAILY. Zhong Nanshan's team responded: Because of the virus isolated from urine,more attention should be paid to the cleanliness of individuals and families. [EB/OL]. https://www.gzdaily.cn/amucsite/web/index.html#/detail/1170150

5.XINHUA DAILY TELEGRARPH. Zhong Nanshan and Li Lanjuan team isolated the virus from the patients' feces [EB/OL]. (2020-02-14)[2020-02-14].http://www.xinhuanet.com/mrdx/2020-02/14/c_1210474176.htm.

6.Y Bai , L Yao , T Wei , *et al*. Presumed asymptomatic carrier transmission of COVID-19. JAMA 2020; published online Feb 21. DOI:10.1001/jama.2020.2565.

Tables

Table:Detection results of different specimens by Real-Time reverse transcriptase–polymerase chain reaction																
location	specimen	feces			pharyngeal swab			urine			hand swab			saliva		
hospital		n	positive(n)	positive(%)	n	positive(n)	positive(%)	n	positive(n)	positive(%)	n	positive(n)	positive(%)	n	positive(n)	positive(%)
	inpatient	24	11	45.8	35	18	51.4	49	9	18.4	34	7	20.6	57	7	12.3
	covert infector	7	4	57.1	11	3	27.3	4	0	0	4	0	0	13	0	0
	total	31	15	48.4	46	21	45.6	53	9	17	38	7	18.4	70	7	10
isolation spot	discharge patient	73	15	20.5	73	0	0	37	0	0	/	/	/	/	/	/
	discharge covert infector	20	3	15	20	0	0	10	0	0	/	/	/	/	/	/
	total	93	18	19.4	93	0	0	47	0	0	/	/	/	/	/	/

Figures

Clinical Type	February															March																					April	
	5	8	9	10	11	15	16	17	18	19	21	24	27	28	29	1	3	4	6	7	9	10	11	12	13	15	16	17	18	19	21	23	25	26	27	28	29	31	1	2
patient1	◎	Δ									※																		★			☆					√			
patient2								◎	Δ							※											★		☆				√							
patient3				◎		Δ						※										★			☆								√							
patient4							◎	Δ								※																			★			☆		√
patient5				◎	Δ							※									★		☆									√								
patient6		◎	Δ											※							★				☆							√								
patient7									◎	Δ							※						★						☆				√							
patient8		◎			Δ							※												★	☆										√					
patient9						◎	Δ												※										★			☆					√			
patient10						◎		Δ							※												★				☆				√					
patient11		◎	Δ												※																★		☆					√		
patient12		◎			Δ										※												★				☆							√		
patient13				◎	Δ							※																				★			☆					√
patient14		◎		Δ										※				★		☆						√														
patient15							◎	Δ								※																		★			☆			√
covert infector1										Δ							※											★	☆					√						
covert infector2									Δ									※											★			☆					√			
covert infector3									Δ				※												★					☆						√				

◎:Symptom onset; Δ: First pharyngeal swabs test positive; ※:Discharge from hospital; ★Last positive feces test
☆:First negative after continuous positive of feces ; √Leave the isolation spot

Figure 1

Time point of result from pharyngeal swabs and feces through the course of 15patient and 3 covert infector with SAR-CoV-2 RNA positive,February to April.