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Reinfection with SARS-CoV-2 in Afghanistan: A case study

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Abstract

Background: One of the critical concerns about the COVID-19 pandemic caused by SARS-CoV-2 is how long the host is protected from reinfection after the first infection. Here we report an individual with two instances of SARS-COV-2 infection.

Methods: A 26-year-old man who has a resident of Kabul, Afghanistan, presented to Afghan Japan Communicable Diseases Hospital on two occasions with symptoms of viral infection and had RT-PCR-confirmed SARS-CoV-2 infection on 16/06/2020. Fourteen days after the initial test, the patient tested positive, again confirmed by RT-PCR results on 30/06/2020, in the patient's isolation, symptoms determined he continued to feel well. However, after 91 days, on October 14, 2020, he tested positive for reinfection to SARS-CoV-2. ELISA (enzyme-linked immunosorbent assay) was performed to detect antibodies in the blood.

Results: A 26-year-old patient was reported with two SARS-CoV-2 positive test results within 91 days. The first positive test was reported on June 16, 2020, and the second positive test (reinfection) was reported on October 14, 2020. An immunoassay analysis in the second infection showed a positive result of IgG and IgM that confirms the availability of disease in the patient's body. It was found that the second infection was symptomatically more severe than the first infection.

Conclusion: Based on the results obtained from RT-PCR and Immunoassay analysis, we found that the patient had two positive SARS-COV-2 tests. However, the genetic confirmation of the spacemen obtained from the first and second infections remains unknown.

Keywords: RT-PCR (Real-Time Polymerase Chain Reaction), ELISA (enzyme-linked immunosorbent assay), COVID-19, Reinfection.

Introduction

The pandemic caused by novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has raised many questions which need to be answered. One of the key concerns about the COVID-19 pandemic by SARS-CoV-2 is how long the host is protected from reinfection. It has been reported that Infection with SARS-CoV-2 generates neutralizing antibodies in patients. **[1]** However, it is not clear how long the immunity acquired during the first infection can protect an individual from reinfection with SARS-CoV-2. Studies show that immunity to other coronaviruses lasts about 1-3 years. **[2,3,6,7,8,9]** Of the reinfection cases reported from Nevada USA **[10]**, Hong Kong **[11]**, Belgium **[12]**, and Ecuador **[13]**, none of the individuals had known immune deficiencies. This indicates that the reinfection is not due to comprised immune response. The reinfection cases where both first and second infections happen due to the same organism can be elucidated as different infection events by genetic analysis of the organism associated with the infection. **[10]** Here, we report the first case of an individual having reinfection to COVID-19 in Afghanistan.

The infections were confirmed based on available diagnostic facilities (Reverse transcriptase-polymerase in reaction-RT-PCR). This case report adds to rapidly growing evidence of COVID-19 reinfection cases; however, the genetic confirmation of the spacemen obtained from the first and second infections remains unknown.

Methods

Case history: Recently, a patient tested positive for SARS-CoV-2 using reverse transcription-polymerase chain reaction in an Afghan Japan hospital, despite earlier recovery from coronavirus 2019 (COVID-19).

Here we present a report of a 26-year-old man from Kabul, Afghanistan, who works as a cleaner in the Afghan Japan hospital. He had no known immune or clinical disorders and had a PCR-confirmed SARS-CoV-2 infection on 16/06/2020. Fourteen days after the initial test, the patient tested positive again, confirmed by RT-PCR results on 30/06/2020) **(Table 1)**. The patient reported diarrhea, nausea, cough, headache, and sore throat symptoms. He recovered in quarantine, testing negative by RT-PCR on 20/07/2020 **(Table 1)**. During the patient's isolation, symptoms were determined, and he continued to feel well. However, after 91 days on the date of October 14, 2020, he was admitted to the emergency unit of Afghan Japan hospital with self-reported severe symptoms of headache, cough, fever, nausea, and diarrhea, and tested positive for reinfection SARS-CoV-2 **(Table 1)**. This case was followed up under the inspection of the technical head of the Afghan Japan hospital laboratory. Ethics approval was waived by the Afghan Japan Hospital and Spinghar Institute of Higher Education, the ethics committee (code: 1386-1407). The patient has provided written consent and has no objection to publishing this report.

Table 1: SARS-CoV-2

	Specimen A			**Specimen B**	
	June 16, 2020	July 13, 2020	July 20, 2020	October 14, 2020	October 15, 2020
Test methodology	Real-time RT-PCR	Real-time RT-PCR	Real-time RT-PCR	Real-time RT-PCR	Immunoassay (IgM and IgG antibody detection)
Test result	Positive	Positive	Negative	Positive	Positive
Quantitative result	Ct 33	Ct 34		Ct 29	

Procedure

Nasopharyngeal swab specimens were obtained from the patient during recovery and isolation in the hospital. The swab was transported to the Afghan Japan Hospital Laboratory in viral transport medium (VTM). Specimens were transported in an ice pack box and stored in refrigeration (4-8℃) for no more than 72 hours before the RNA extraction and subsequent real-time RT-PCR.

RNA isolation and Real-Time- PCR:

Total RNA was isolated from the sample; the Specimens were preserved in viral transported media (VTM) and tested within 24 hours. Then RNA was extracted with the (Qiagen QIAamp Viral RNA Mini Kit extraction method). Extracted RNA from all thespecimens kept at -20 ℃ in the refrigerator.

Preparation of reagents Master-mix :

The 2019-nCoV-PCR Master Mix (26 µL 2019-nCoV-PCR Mix +4 µL 2019- nCoVPCR-Enzyme Mix) was prepared based on the total number of specimens 2019-nCoV-PCR-Positive Control and 2019-nCoV-PCR-Negative Control and were mixed thoroughly **(Table 2)**. Next, cDNA was synthesized, and DNA was amplified using an amplification kit (SUNSURE BIOTECH) as per the manufacturer's instruction and with an elution volume of 50 µl. For running a real-time RT-PCR (rotor gene q 5 plex) for amplification, the PCR cycling program was performed as follows: 30 min at 50 ℃ and cycle 1 for reverse transcription, 1 min at 95 ℃ and cycle 1 for cDNA pre-denaturation, 15 sec at 95 ℃ and 30 sec at 60 ℃ cycles 45 for denaturation and Annealing, extension and fluorescence collection, finally 10 sec at 25℃ and a single process for device cooling **(Table 3)**. For SUNSURE BIOTECH (Amplification kit), RT-PCR, the threshold for calling a specimen positive is a CT value of less than 35.

Table 2: Master mix preparation:

	1 sample	**10 sample**	**24 sample**	**48 sample**
2019-nCoV-PCR Mix(µl)	26	260	624	1248
2019-nCoV-PCR-Enzyme Mix(µL)	4	40	96	192
Note: The above configuration is just for your reference, and to ensure enough volume of the PCR-Master-mix, more importance on the actual pipetting may be required.				

Table 3: set cycle parameter for RT-PCR:

	Steps	**Temperature**	**Time**	**Cycle**
1	Reverse Transcription	50 ℃	30 min	1
2	cDNA predenaturation	95 ℃	1 min	1
3	Denaturation	95 ℃	15 sec	45

	Annealing, extension, and fluorescence collection	60 ℃	30 sec	
4	Device Cooling	25℃	10 sec	1

Immunoassay

ELISA was performed to detect antibodies in the blood using (Automatic Chemiluminescence Immunoassay Analyzer Acre) ELISA machine and (2019-nCoV IgM) regents **(Table 1)**. By applying the RT-PCR technology, this test utilizes the novel coronavirus (2019-nCoV) ORF 1ab and the specific conserved sequence of coding nucleocapsid protein N gene as the target regions designed for conserved the double-target genes to detect the sample RNA via fluorescence signal changes.

Results

The first nasopharyngeal swab, obtained in Afghan Japan infectious diseases hospital (specified for COVID-19 patients) on June 16, 2020, was positive for SARS-CoV-2 on real-time RT-PCR testing. Fourteen days later, the patient tested positive again. Subsequent nucleic acid amplification tests were negative for SARS-CoV-2 RNA after the resolution of symptoms. The patient's symptoms returned before Oct 14, 2020. He was admitted to the hospital, and a second nasopharyngeal swab was obtained and was positive for SARS-CoV-2 infection by real-time RT-PCR testing.

The patient's symptoms appeared to be more severe than the first infection, including myalgia, cough, shortness of breath, headache, fever, and diarrhea. On October 15, 2020, the patient was tested for IgG and IgM against SARS-CoV-2, and positive results were obtained **(Table 1)**. The CT (cycle threshold) value for first and reinfection resulted in 33 and 29, as shown in **Table 1**.

Discussion

We report the first individual in Afghanistan to have symptomatic reinfection with SARS-CoV-2. Similar cases have been reported in Nevada, USA 10, Hong Kong **[11]**, Belgium **[12]**, and Ecuador. **[13]** In our case and the case reported in the USA, the patients showed increased symptom severity in their second infection. In contrast, the cases from Belgium, Netherlands **[12]**, and Hong Kong **[11]** did not show severe symptoms in the second infection.

There can be various reasons for the reinfection. First, a very high dose of the virus might have led to the second instance of infection and induced a more severe disease.**[14]** Second, it is possible that reinfection was caused by repeated exposure to the virus as the patient was working in a COVID-19 specified hospital and was dealing with COVID-19 infected patients. Third, a mechanism of antibody-dependent enhancement can be the reason for reinfection where specific Fc-bearing immune cells like monocytes and macrophages become infected with the virus by binding to specific antibodies. This mechanism has been described previously with the betacoronavirus causing the severe acute respiratory syndrome. 15Deactivation and reactivation of the virus can be another possible reason for reinfection. However, to prove this hypothesis, the mutation rate of SARS-CoV-2 needs to be elucidated, which has not yet been done. **[16,17,18,19]**

The patient had no immunological disorders; this indicates that the reinfection is not due to comprised immune response. A significant limitation of our case study is the unavailability of genomic analysis data of the first and second infection. Due to the unavailability of gene sequencers and phylogenic analysis equipment, we could not identify the viral sequence of the first and second episodes of SARS-CoV-2 infection. Thus, we could not confirm if the first and second infections were due to the different variants of the same viruses or if there was any other reason behind it. Additionally, we could not assess the immune response in the first episode of SARS-CoV-2 infection. Also, the immune response's effectiveness (e.g., neutralizing antibody titers) during the subsequent infection was unknown when the patient was antibody positive for SARS-CoV-2 nucleocapsid protein. If our case study denotes reinfection, it is essential to identify the frequency of such cases because it is rare, and we cannot rely on a single case to prove the reinfection. Genomic sequencing of positive cases in Afghanistan and worldwide can provide more information to find and confirm reinfection cases.

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Data availability statement: The raw data supporting the conclusions of this article will be made available by the authors, on reasonable request to the corresponding author.

Competing interests: All authors declared no potential personal or financial conflicts of interest.

Ethics statement: This study was ethically approved by the medical bioethics committee of the SIHE ethics committee (code: 1386-1407). The patients/participants provided their written informed consent to participate in this study.

Author contributions: HS, and STP were involved in the study's conception, design, statistical analysis, and interpretation of the data. SUZ, AMB, and AS were involved in data collection, data cleaning,

statistical analysis, and manuscript drafting. AMB supervised the study. All authors approved the final manuscript for submission.

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Key Points

- We report the first case in Afghanistan where an individual is infected twice with SARS-COV-2.
- This case report adds to rapidly growing evidence of COVID-19 reinfection cases.
- This case and similar studies would have implications for the role of vaccination in response to COVID-19.
- If we have truly reported a case of reinfection, this indicates that the initial infection with SARS-COV-2 cannot provide absolute protection for the individual from subsequent infection.
- The patient's symptoms in the reinfection seemed more severe than in the first infection.

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