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Sequential HCoV-HKU1 and SARS-CoV-2 Infections, a Case Report

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Case Report

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ABSTRACT

In the SARS-CoV-2 pandemic, the seasonal viral respiratory infections had a minimum prevalence due to public health precautions to reduce the risk of getting Coronavirus Disease 19 (COVID-19). There have been reports of COVID-19 coinfection with influenza, respiratory syncytial virus (RSV), and seasonal coronaviruses during the pandemic. Here, we report a case in which the patient had sequential respiratory infections of human coronavirus HKU1 (HCoV-HKU1) and SARS-CoV-2 in a fully vaccinated, healthy person. It should be noted that other seasonal coronaviruses that could cause symptomatic RTIs might be misdiagnosed clinically with COVID-19. Hence, we highly recommend monitoring and follow-up of symptomatic patients with negative SARS-COV-2 RT-PCR results.

INTRODUCTION

From late 2019 to early 2020, all the countries throughout the world were gradually affected by a severe acute respiratory syndrome (SARS) infection termed in the following COVID-19. Its etiologic agent is a new coronavirus known as SARS-CoV-2, which is an enveloped and a positive-sense single-stranded RNA virus [1]. Several variants have emerged despite the vaccination and some therapeutic regimens against SARS-CoV-2. The emergence of new mutations and, more importantly, new variants of concern (VOCs) was predictable due to the high rate of viral replication and infection of many people worldwide, resulting in more widespread and faster distribution of SARS-CoV-2.

All the four seasonal human coronaviruses, including HCoV-229E, HCoV-OC43, HCoV-NL63, and HCoV-HKU1, can cause infection in the respiratory tract and cause RTIs. The pre-existing immunity to these seasonal coronaviruses might last for up to 12 months, and re-infection with the same or different strains is expected [3]. There are reports of co-infection of human coronaviruses like HCoV-HKU1 with other viruses, including SARS-CoV-2 and influenza A virus [4, 5]. A meta-analysis of cases in the Netherlands, Belgium, and Spain showed 5.3% and 9.1% COVID-19 co-infection with influenza in men and women [6]. The influenza virus detection rate has substantially fallen to ~1% from April 2020 compared to the previous years. During the ongoing COVID-19 pandemic, even one of the influenza B virus lineages named B/Yamagata/16/1988 vanished [7]. Another study revealed 0.0015, 0.0028, and 0.0169 times reduction in positive tests of influenza A, B, and RSV, respectively, compared to the pre-pandemic levels [8].

It is not surprising that the burden of other RTIs has dramatically decreased in the current COVID-19

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pandemic, contributing to public health countermeasures like hand washing, mask-wearing, keeping social distance, and travel restrictions [7]. However, detecting influenza and common cold viruses such as endemic human coronavirus and their potential role in intensifying or abating COVID-19 has received attention. In addition to SARS-CoV-2 re-infection, co-infection and sequential infections with other respiratory viruses have occurred in some cases [4-6].

CASE REPORT

On Monday, September 13, 2021, a 30-year-old female healthcare worker presented to the Bank Melli Iran Hospital, Tehran, Iran, with severe sneezing, runny nose, hot flashes, and eye irritation. She was febrile and suffering from severe backache and neck pain. She had a history of taking public transportation to travel from Tehran to Lorestan Province, and she had social contact with her family from the previous Friday to the following Sunday, September 12, 2021. Nasopharyngeal and oropharyngeal swabs were obtained from her and tested by two COVID-19 rapid antigen kits (LUNGENE, China, and HUMASIS, Republic of Korea) and Real-Time PCR (Pishatzteb, Iran) assays. The results of all tests were negative. On Thursday, September 14, 2021, all signs and symptoms continued, with the addition of myalgia, backache, and neck pain, and the results of the rapid and Real-Time PCR tests were negative for SARS-CoV-2 once more. On Wednesday, September 15, 2021, she experienced a sore throat, inflammatory joint pain, osteodynia, and previous complications. She was referred to the COVID-19 National Reference Laboratory (CNRL), Pasteur Institute of Iran, and nasopharyngeal and oropharyngeal swabs were taken and tested by a different SARS-CoV-2 Real-Time PCR kit (Sansure Biotech, China). The Real-Time PCR test yielded a negative result. Myalgia and backache were relieved two days later, on Friday, September 17, 2021. However, severe sinusitis, digestive complications such as diarrhea, and heart palpitations developed. Nasopharyngeal and oropharyngeal swabs collected on Friday, 17, 2021, tested positive by SARS-CoV-2 Real-Time (Pishatzteb, Iran) in the Bank Melli Iran hospital with Ct values: 13 for ORF1ab and 14 for E gene. The samples were retested in CNRL using another Real-Time PCR assay (Sansure Biotech, China), and the positive result was confirmed (Ct Values: ORF1-ab: 17, N: 16). She began using a sinus wash solution and taking single-pill daclatasvir/sofosbuvir (60mg/400mg, SOVODAK), prescribed by her physician. All disease complications were alleviated from Friday, but septic sputum and nasal discharge lasted for another week. Eight days after the first positive test, she was cleared on Saturday, September 25, 2021, and her SARS-CoV-2 Real-Time PCR test turned negative. Her backache and mild diarrhea persisted for nearly two days after the negative test. She took single-pill sofosbuvir and daclatasvir for a whole ten-day regimen. It is noteworthy that she had received two doses of the Sputnik V vaccine (Gam-COVID-Vac) about six months ago. Her SARS-CoV-2 anti-S1 IgG was measured by a commercial ELISA kit (Euroimmun, Germany) about two weeks before symptoms onset and tested positive (signal to cutoff ratio, S/CO: 1.42, a little higher than borderline of positivity, S/CO 1.1).

Additionally, all samples were tested by HiTeq 17 Viro Respiratory Pathogen, One-Step RT-PCR kit (GeneovA, Iran), a multiplex Real-Time PCR assay for identification of 17 respiratory viruses, including SARS-CoV-2, Influenza A, Influenza H1N1, Influenza B, HCoV-HKU1, HCoV-OC43, HCoV-NL63, HCoV-229E, Metapneumovirus, Respiratory Syncytial Virus, Human Bocavirus 1-3, Human Parainfluenza 1-3, and Adenovirus. In line with SARS-CoV-2 Real-Time PCR tests, SARS-CoV-2 RNA was detected in the fourth sample obtained on September 17, 2021. Interestingly, the multiplex assay showed that the first, second, and third samples (obtained on September 13, 14, and 15, 2021) were positive for HCoV HKU1/ HCoV OC43. To confirm HKU1/ HCoV OC43 infection, a pan coronavirus RT-PCR assay was employed to amplify about 600 bp of the genome [9]. The PCR products were subsequently sequenced with the amplification primers via the Sanger assay by a 3130 Genetic Analyzer (Thermo Fisher Scientific, USA). The raw data of sequencing was trimmed and assembled using CLC Main Workbench software (CLC bio, Denmark) and then confirmed by BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The primers utilized for amplification and sequencing are listed in Table 1.

Table 1. The primers used for the sequencing of viruses.

Primer	Target	Forward Sequence	Reverse Sequence
SARS-CoV-2 A	Spike	GGTACTGCTGTTATGTCTTTAAAAG	ACAATAAGTAGGGACTGGGTC
SARS-CoV-2 B	Spike	GAGAAGTCTAACATAATAAGAGGCTG	GTCTACAGCATCTGTAATGGTTC
SARS-CoV-2 C	Spike	TCTTCAGGTTGGACAGCTG	CGGCCTGATAGATTTCAGTTG
SARS-CoV-2 D	Spike	GAGAATCAGCAACTGTGTTG	GACTCAGTAAGAACACCTGTG
SARS-CoV-2 E	Spike	GGACCAGCAACTGTTTGTG	TTTCTGCACCAAGTGACATAG
SARS-CoV-2 F	Spike	ATATGAGTGTGACATACCCATTG	ACAAATGAGGTCTCTAGCAGC
HCoV-HKU1	RdRP	GGTTGGGATTATCCTAAATGTGA	TGTTGAGAGCAAAATTCATG

The sequencing result showed infection with HCoV-HKU1 during the early days of symptomatic illness (GenBank Accession Number, OL679306) and with the Delta variant of SARS-CoV-2 in the following days of symptomatic illness (GISAID submission ID,

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EPI_ISL_6799579). However, after a positive test for SARS-CoV-2, the Real-Time PCR result did not detect HCoV-HKU1 anymore. Interestingly, the sample obtained on Friday became negative for other respiratory viruses. In other words, the HCoV-HKU1 infection was cleared when her COVID-19 appeared. Indeed, the SARS-CoV-2 and HCoV-HKU1 co-infection seem unlikely, and it appears that HCoV-HKU1 infection occurred first, followed by SARS-CoV-2 infection. Table 2 represents the sampling date and the diagnosed infections. On November 15, 2021, the chest X-ray findings showed no crucial abnormalities; both lungs were clear with no infiltrates, and the heart size was normal with no evidence of a mediastinal shift (Fig. 1).

The Ethical Committee of Pasteur Institute Iran approved this study (ethical code: IR.PII.REC.1399.073)

Table 2. The timepoint table of the patient's history and the RT-PCR results.

Date of Sampling	SARS-CoV-2 infection	HCoV HKU1 infection
Monday, 13 Nov. 2021	Negative	**Positive**
Tuesday, 14 Nov. 2021	Negative	**Positive**
Wednesday, 15 Nov. 2021	Negative	**Positive**
Friday, 17 Nov. 2021	**Positive**	Negative
Saturday, 25 Nov. 2021	Negative	Negative

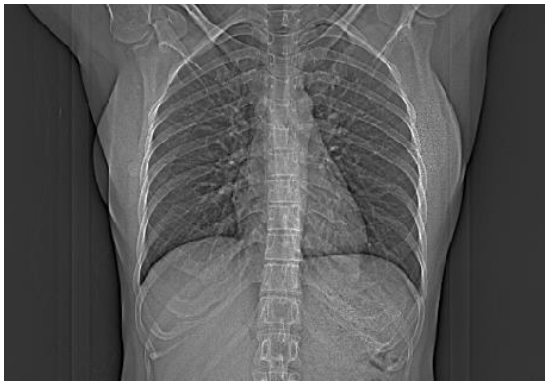

Fig. 1. The chest X-ray image of the patient with respiratory tract infection's symptoms. No infiltrates or mediastinal shifts were seen, and heart size was normal.

DISCUSSION

COVID-19 co-infection or superinfection with other respiratory pathogens is a severe public health challenge, especially when the main culprit, i.e., COVID-19, overstretches the health system. Additionally, outbreaks of other respiratory viruses in hospital settings could lead to harmful situations. Hospitalization of clinically suspected COVID-19 cases with negative PCR tests but with other viral infections like common coronaviruses or influenza virus in COVID-19 ICU may facilitate transmitting the infection to actual COVID-19 patients and exacerbate their condition. On the other hand, the transmission of COVID-19 to the non-infected but vulnerable patients may happen. In the first year of the COVID-19 pandemic, several outbreaks of other respiratory viruses were recorded in 2021. Therefore, a syndromic approach in laboratory diagnosis would be essential in controlling the outbreaks and adequately managing the COVID-19 cases.

A general prevalence of respiratory viruses responsible for RTIs in the pre-COVID-19 era was between 13-59%, 1-36%, 1-56.8%, and 0.5-18.4% for rhinoviruses, human adenoviruses, human bocaviruses, and seasonal coronaviruses, respectively [10]. Non-pharmaceutical countermeasures for COVID-19 during the first year of the pandemic, such as mask-wearing, social distancing, and curfews, resulted in the significant reduction of other respiratory viruses [11, 12]. However, several studies have demonstrated a variable co-infection rate between SARS-CoV-2 and other respiratory pathogens, with the most prevalent agents being influenza and rhinovirus [6, 13]. Meanwhile, some studies reported the co-infection of SARS-CoV-2 and seasonal coronaviruses [4, 10].

It is unclear whether the patient acquired HCoV-HKU1 and SARS-CoV-2 infections coincidently but with symptoms onset appearing at different times or if the exposure to HCoV-HKU1 and SARS-CoV-2 were sequential. Whether the coinfection of HCoV-HKU1 and SARS-CoV-2 had synergy or interference with each other remains a controversy. The limitations of this study were the absence of immune history against HCoV-HKU1. Immune memory against seasonal coronaviruses might help prevent a severe form of COVID-19. Dugas *et al.* (2021) observed that patients with a lower concentration of antibodies against HCoV-OC43 and HCoV-HKU1 presented a severe form of COVID-19 [14].

There are some differences in the chest CT scans between patients infected with the influenza A virus and

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COVID-19. However, the rest of the chest CT findings were not significantly different between them [15]. On the other hand, a study revealed that the chest CT findings of SARS-CoV-2 and endemic HCoV community-acquired pneumonia (CAP) are dissimilar, especially in the ground-glass opacities (GGOs) pattern, which distinguish these two CAP from each other [16]. However, this issue remains controversial yet [17].

It is important to note that the abovementioned case is a healthcare worker and health centers are usually among the main transmission settings for infectious agents. Therefore, she had a higher risk of getting various respiratory infections, and the higher risk of co-infection or sequential infection with other respiratory infectious agents is highly expected. Also, the relatively same symptoms result in the misdiagnosis of diseases caused by various viral respiratory infections.

Considering the high frequency of genetic recombination among coronaviruses, concurrent circulation of SARS-CoV-2 and other coronaviruses like HKU-1 observed in this study could potentially lead to recombination and emerging phenotypically different viruses. When two coronaviruses infect the same cell simultaneously, copy-choice recombination may occur during RNA synthesis. In this template switching phenomenon, the RNA-dependent RNA polymerase (RdRp) and nascent strand dissociate from the template strand and restart in different template regions or another new template [18, 19]. This phenomenon might have happened to the fifth SARS-CoV-2 variant of concern (VOC), Omicron variant, or B.1.1.529, designated by WHO on November 26, 2021 [20]. Comparing the Omicron variant spike and the other recorded SARS-CoV-2 genomes showed a unique insertion of three amino acids in the Omicron variant spike (ins214EPE) possibly acquired during co-infection with HCoV-229E [21].

Learning points:

- The SARS-CoV-2 is not the only spreading virus anymore.
- The probability of coinfection or sequential infection of SARS-Cov-2 and other respiratory viruses has increased.
- The close sequential infections and coinfections might increase the recombination chance.

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CONFLICT OF INTEREST

The authors declare there are no conflicts of interest associated with this manuscript.

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