Epidemiology and Infection

cambridge.org/hyg

COVID-19 re-infection in Shahroud, Iran: a follow-up study

Fariba Zare[1], Maryam Teimouri[2], Ahmad Khosravi[3], Marzieh Rohani-Rasaf[3], Reza Chaman[3], Ali Hosseinzadeh[3], Hozhabr Jamali Atergeleh[3], Ehsan Binesh[4] and Mohammad Hassan Emamian[5]

[1]Center for Health Related Social and Behavioral Sciences Research, Shahroud University of Medical Sciences, Shahroud, Iran; [2]Department of Biochemistry, School of Allied Medical Sciences, Shahroud University of Medical Sciences, Shahroud, Iran; [3]Department of Epidemiology, School of Public Health, Shahroud University of Medical Sciences, Shahroud, Iran; [4]Clinical Research Development Unit, Imam Hossein Hospital, Shahroud University of Medical Sciences, Shahroud, Iran and [5]Ophthalmic Epidemiology Research Center, Shahroud University of Medical Sciences, Shahroud, Iran

Short Paper

Cite this article: Zare F, Teimouri M, Khosravi A, Rohani-Rasaf M, Chaman R, Hosseinzadeh A, Jamali Atergeleh H, Binesh E, Emamian MH (2021). COVID-19 re-infection in Shahroud, Iran: a follow-up study. *Epidemiology and Infection* **149**, e159, 1–4. https://doi.org/10.1017/S095026882100087X

Received: 9 February 2021
Accepted: 2 March 2021

Key words:
COVID-19; incidence; re-infection; SARS-COV

Author for correspondence:
Reza Chaman,
E-mail: rezachaman@yahoo.com

Abstract

Although many people became infected and recovered during the COVID-19 epidemic, the immunity duration and re-infection in recovered patients have recently attracted many researchers. The aim of this study was to evaluate the recurrence of the infection in recovered individuals over a 9-month period after the onset of the COVID-19 epidemic. In this study, data related to COVID-19 patients in Shahroud city were collected using the electronic system for registering suspicious patients and also by checking patients' hospital records. In this study, from 20 March 2020 to 20 November 2020 (9 months), a total of 8734 suspected patients with respiratory symptoms were observed and followed up. RT-PCR was positive for 4039 patients. During this period, out of the total number of positive cases of COVID-19, 10 cases became re-infected after complete recovery. The risk of re-infection was 2.5 per thousand (0.95 CI 1.2–4.5). The mean time interval between the first infection and re-infection was 134.4 ± 64.5 days (range 41–234 days). The risk of re-infection between male and females was not statistically different (1.98 per 1000 women and 2.96 per 1000 men). Exposure to COVID-19 may not establish long-term protective immunity to all patients and may predispose them to re-infection. This fact can be reminded that the use of masks, social distancing and other preventive measures are very important in recovered patients and should be emphasised especially in health care personnel who are more exposed to the virus.

Introduction

The first case of COVID-19 was discovered in Wuhan, China in December 2019 and soon spread around the world [1]. To date (28 January 2021), worldwide, more than 100 million people have been infected and nearly 2.180 million have died [2]. Now, almost a year after the start of the COVID-19 pandemic, there are still many ambiguities and questions about it. One question that has always been associated with SARS CoV-2 is whether there is a possibility of re-infection following initial infection and after complete recovery of the patient. In many studies to report re-infection rate, different numbers were reported to speculate the interval between the first infection and re-infection, with some reported 20–22 days for complete removal of virus from patient's body [3–5]. In others, a very short interval was considered for re-infection, which may not be considered as re-infection at all and still must be considered as the primary infection [1, 6, 7]. Due to various duration of the immune response in the body and the reporting of different time intervals due to differences in age and sex of COVID-19 patients, a precise definition of re-infection is not yet available.

According to the World Health Organization definition, two negative tests 24 h apart were considered as a cure for COVID-19. Assuming that the immune response is incomplete following a normal viral infection and re-infection is possible, re-infection can be defined as clinical recurrence of COVID-19-compatible symptoms with a positive PCR test. Although many studies have been conducted in China [8–10], Korea [11], USA [6] and Brazil [12] on this matter, however, the available evidences are not enough and further studies can provide more accurate statistics about re-infection.

In addition, the number of these studies in Iran is very small, and since the severity and rate of re-infection may vary in different societies depending on the type and rate of immune response, our aim in this study was to evaluate the rate of re-infection over a 9-month period after the onset of COVID-19 epidemic in Shahroud, a city located in northeastern Iran.

CAMBRIDGE UNIVERSITY PRESS

Methods

The Shahroud is located in northeastern Iran with a population of 285 000 [13]. COVID-19 patients are admitted to a referral hospital in the area, and there are 96 health centres to record and follow-up on COVID-19 cases, both outpatients and inpatients.

In this study, COVID-19-confirmed cases were those who presented clinical symptoms of COVID-19 and their RT-PCR test was positive based on their nasopharyngeal and pharyngeal specimens. The criterion for re-infection was to have clinical symptoms with SARS-CoV-2 PCR-positive test at least 30 days after first positive test. This criterion was based on the findings of studies that reported that the dosage and load of virus would get to its minimum after 28 days [14, 15].

Clinical and demographic information of patients is recorded and followed in a comprehensive electronic system for surveillance, prevention and treatment of COVID-19 in this area. The information required in this study was extracted using this electronic system and also by referring to the patients' hospital records. The protocol related to this study has been proposed in the ethics committee of Shahroud University of Medical Sciences, and has been approved under the number IR.SHMU.REC.1398.160.

Results

In this study, from 20 March 2020 to 20 November 2020 (9 months), a total of 8734 patients with suspected respiratory symptoms were taken care and followed up. RT-PCR was positive for 4039 of these patients. Out of the total number of these COVID-19 patients, 1849 patients (45.8%) were hospitalised and 2190 patients (54.2%) were treated on an outpatient basis. The mean age of patients was 64 ± 28 years ranging from 13 to 90. Approximately half of the patients and hospitalisations were among men, 2025 (50.2%) and 902 (48.8%), respectively.

Health status and symptoms among patients were followed up after recovery. Among the patients followed, 49 tested positive for RT-PCR after recovery. Of these, 39 were excluded due to repeated testing at 1-month interval. Most of these individuals were retested to ensure a positive test or repeat the test in both outpatient and inpatient settings during the course of the illness, which excluded re-infection cases. Therefore, the remaining 10 patients who had recurrent clinical signs and positive PCR test were considered as definitive cases of COVID-19 re-infection after a period of complete recovery.

The incidence of re-infection in this study was estimated to be 2.5 per thousand patients, which was 1.98 per thousand in women and 2.96 per thousand in men. The risk of re-infection between male and females was not statistically different. The mean time interval between the first infection and re-infection was 134.4 ± 64.5 days (range 41–234 days). Out of these 10 definitive re-infected patients, four were female and six were male (Table 1). Four of them were admitted to the intensive care unit both in primary infection and re-infection period and four were referred and treated on an outpatient basis on both periods. Two of them had mild symptoms in the primary stage but re-infection was severe for them or *vice versa*. Three medical staffs (physician and nurse) were among the patients with re-infection who worked in the referral hospital of the area. Four of them had died at the hospital due to COVID-19. According to the hospital records of the deceased, all of them were over 80 years old and all of them had one or more underlying diseases including heart disease, diabetes, gastrointestinal bleeding, fractures, or a history of surgery and lung diseases.

Table 1. Characteristics of 10 COVID-19 re-infection patients

Sample date (1)	Sex	Age-year	Duration of hospitalisation (days)	Discharge date (1)	Sample date (2)	Duration of hospitalisation (says)	Discharge date (2)	Interval between two positive sample test	Outcome
11 March	Male	50	Outpatient	N/A	27 October	Outpatient	N/A	230	Survive
12 March	Female	81	4	16 March	29 October	7	5 November	234	Passed away
31 March	Female	42	Outpatient	N/A	14 September	Outpatient	N/A	107	Survive
31 March	Male	84	12	12 April	19 May	1	20 May	49	Passed away
14 April	Male	90	18	2 May	20 May	Outpatient	N/A	41	Survive
16 April	Male	27	Outpatient	N/A	15 July	Outpatient	N/A	115	Survive
2 May	Male	79	Outpatient	N/A	28 September	16	14 October	150	Passed away
15 May	Male	86	3	18 May	23 October	9	1 November	164	Passed away
4 June	Female	90	21	25 June	10 October	12	22 October	130	Survive
6 July	Female	13	Outpatient	N/A	7 November	Outpatient	N/A	124	Survive

One had both a history of bone marrow cancer and chemotherapy due to leukaemia and also heart disease.

Discussion

Infection of a pathogen in a person leads to its imprint in the immune system, a phenomenon known as immunological memory, which can protect that person from subsequent infection for decades. This occurs through the induction of B and T lymphocytes with antigen-specific memory as well as a persistent antibody response that prevents re-infection [16]. Although re-infection with viruses that cause systemic infections, such as measles, mumps, rubella, hepatitis A virus, is very uncommon, re-infection with viruses that cause mucosal infection without viremia, such as respiratory syncytial virus, influenza and seasonal coronavirus, is common [17]. One of the most important reasons for this is the much longer antibody response in systemic viral infections [18]. Re-infection is seen with many respiratory viruses, including COVID-19 viruses. Re-infection with respiratory viruses may be due to a weakened or diminished primary immune response (e.g. respiratory syncytial virus), re-infection with the genotype of another species (e.g. nasal viruses) or high diversity of viruses (e.g. flu viruses).

The present study showed that 2.5 per thousands of COVID-19 patients develop recurrence of this disease. Reports of COVID-19 re-infection have been published worldwide [6, 10, 19, 20]. But the remarkable point was the younger age range (between 21 and 60 years) that other studies have reported in re-infection of COVID-19 [12, 21, 22]. Due to the fact that seroconversion is observed in most patients with SARS-COV-2, however, the titre of binding and neutralising antibodies is very variable between different individuals and decreases over time [16]. It has also been reported that people with more severe disease have higher levels of neutralising antibody titres, and that antibody levels are still detectable 2–3 months after primary symptoms, while those who were asymptomatic or had mild symptoms had lower antibody titres, and in less than 2 months the antibodies started to decrease [23].

Although one study showed that these antibodies can protect against re-infection for several months after infection, the exact titre of antibodies needed to neutralise the virus to prevent re-infection must be determined [9]. T cells, as another arm of the immune system, play an important role in maintaining long-term immunity against viruses and re-infection. Studies on SARS-CoV-2 and other coronaviruses have shown that coronaviruses can promote long-term T-cell immunity [16]. In another study, SARS-CoV-2-specific T, CD4 and CD8 cells were retained for more than 6 months after initial infection, and these cells mainly detect structural proteins of the virus such as spike, nucleocapsid and membrane proteins of the virus [5].

The rate of antibody response and duration of its durability as well as the duration of cellular immunity vary in different individuals and can play an important role in determining susceptibility to re-infection. On the other hand, in our study, 30% of re-infection cases were from the medical staff who were constantly exposed to the virus and this could be a reason for re-infection. In contrast, re-infection in other patients can be reduced due to adhering safety protocols and the fear created by the previous encounter. Although in this study, milder symptoms were seen in re-infection cases, four out of 10 patients with re-infection showed more severe symptoms and died. In a study by Tillett *et al.* [24], which studied re-infection in a 25-year-old, more severe symptoms were observed in re-infection. More severe re-infection in this study may occur for several reasons: (1) all deceased re-infection cases were old and had an underlying disease, while younger, disease-free re-infected individuals showed a milder range of symptoms. Given that the risk of death is inherently high in the elderly and those with underlying disease, this can be the cause of more severe relapse and death in these individuals. (2) In re-infection, exposure of patients to higher doses and viral loads may have been the cause of more severe infection and death. (3) Re-infection may have been caused by a mutated form of the disease [24, 25].

Reports of recent genetic analyses of SARS-CoV-2 re-infection in two case studies showed that there are genetic differences between primary SARS-CoV-2 and SARS-CoV-2 re-infection [24, 26]. In one study, the results of genomic sequencing showed that the two SARS-CoV-2 viruses were different in 24 nucleotide positions. This finding indicates that the virus strain detected in the second infection was completely different from the strain in the first infection. Therefore, in some cases, re-infection may occur despite the static level of specific antibodies. Genomic sequencing was not performed in the present study.

Due to the importance of these mutations, it has been shown that some mutations reported in re-infection have happened in the spike protein at the binding site of this protein to neutralising antibodies or at the response site of CD4 cells [26–28] and so these protein changes may make the virus less exposed to the neutralising antibodies or CD4 cells that were made in the first infection. Therefore, despite gaining natural immunity in the first infection, re-infection with SARS-CoV-2, like other human coronaviruses causing colds, may be present [26, 28].

One of the strengths of the present study is that we were able to follow-up 4039 positive patients during the 9-month period of the COVID-19 epidemic due to the early launch of an integrated COVID-19 registration system as well as equipping a laboratory for PCR testing and having only one referral hospital, therefore we can be sure that there are almost no re-infections that have not been detected. Also in this study, the laboratory conditions are completely the same and the standards were observed, and on the other hand, all patients, in addition to PCR test, also had typical symptoms of COVID-19, such as fever, cough and shortness of breath, so concerns about the impact of sampling method, sampling location and staff skills on positive or negative test results that have been addressed in some studies [29–31] can be alleviated. Another strength of this study is the early detection of re-infections, which indicates the active diagnosis and success of the patient surveillance system. However, this study also had limitations, one of which was the lack of a definite criterion for recovery in patients, which in order to overcome infected people were excluded from the study a month after the onset of infection. As a final limitation, it can be said that in any society, a percentage of patients, especially at a younger age, do not go to medical centres at all or do not have RT-PCR test. It is natural that the present study did not consider this type of patient and our estimate of re-infection may be slightly underestimated. On the other hand, if serological tests, antibody titration and genomic sequencing could be performed, it could be of great help in finding the cause of re-infection in our study population.

Conclusion

According to the findings of this study, relative immunity develops following COVID-19 infection, nevertheless there is a small possibility of re-infection in people recovering from COVID-19, and the

severity of its re-infection can vary from mild to very severe and eventually may cause death. This fact can be reminded that the use of masks, social distancing and other preventive measures are very important in recovered patients and should be emphasised especially in health care personnel who are more exposed to the virus.

Data. The datasets used during the current study are available from the corresponding author on reasonable request.

Financial support. This work was supported by Shahroud University of Medical Sciences (Grant No. 98126).

Conflict of interest. None.

References

1. **Zhu N *et al.*** (2020) A novel coronavirus from patients with pneumonia in China, 2019. *New England Journal of Medicine* **382**, 727–733. doi: 10.1056/NEJMoa2001017
2. **World Health Organization** (2020) WHO coronavirus disease (COVID-2019) dashboard. Available at: https://www.who.int/emergencies/diseases/novelcoronavirus- (Accessed 28 January 2020).
3. **Tang Xiao A *et al.*** (2020) Dynamic profile of RT-PCR findings from 301 COVID-19 patients in Wuhan, China: a descriptive study, *Journal of Clinical Virology* **127**, 104346. doi: https://doi.org/10.1016/j.jcv.2020.104346
4. **Zhou F *et al.*** (2020) Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *The Lancet* **395**, 1054–62. doi: https://doi.org/10.1016/S0140-6736(20)30566-3
5. **Dan JM *et al.*** (2021) Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. *Science* **371**(6529), eabf4063. doi: 10.1126/science.abf4063.
6. **Duggan NM *et al.*** (2020) Is novel coronavirus 2019 reinfection possible? Interpreting dynamic SARS-CoV-2 test results. *The American Journal of Emergency Medicine(AJEM)* **39**, 256.e1-.e3. doi: https://doi.org/10.1016/j.ajem.2020.06.079
7. **Luo A** (2020) Positive SARS-Cov-2 test in a woman with COVID-19 at 22 days after hospital discharge: A case report. *Journal of Traditional Chinese Medical Sciences* **7**, 413–417. doi: https://doi.org/10.1016/j.jtcms.2020.04.001
8. **Ai T *et al.*** (2020) Correlation of Chest CT and RT-PCR Testing for Coronavirus Disease 2019 (COVID-19) in China: A Report of 1014 Cases. *Radiology* **296**, E32–E40. doi: 10.1148/radiol.2020200642
9. **Lumley SF *et al.*** (2020). Antibodies to SARS-CoV-2 are associated with protection against reinfection. (preprint) *medRxiv*.
10. **Zheng KI *et al.*** (2020). A Case Series of Recurrent Viral RNA Positivity in Recovered COVID-19 Chinese Patients. *Journal of General Internal Medicine* **35**, 2205–2206. doi: 10.1007/s11606-020-05822-1
11. **Kang YJ and Joo H** (2020) South Korea's COVID-19 infection status: From the perspective of reconfirmation after complete recovery. *Journal of Pure and Applied Microbiology* **14**, 1073–1075.
12. **Bonifácio LP *et al.*** (2020) Are SARS-CoV-2 reinfection and Covid-19 recurrence possible? a case report from Brazil. *J Revista da Sociedade Brasileira de Medicina Tropical* **53**, e20200619. http://dx.doi.org/10.1590/0037-8682-0619-2020
13. **Statistical Center of Iran** (2016) Results of the general population and housing census 2016, N.S.C.o. Iran, Editor. Available at https://www.amar.org.ir/english/Population-and-Housing-Censuses
14. **Shaikh A *et al.*** (2020) Prolonged SARS-CoV-2 viral RNA shedding and IgG antibody response to SARS-CoV-2 in patients on hemodialysis. *Clinical Journal of the American Society of Nephrology (CJASN)* **16**, 290–292. doi: 10.2215/CJN.11120720 %J.
15. **Agarwal V *et al.*** (2020) Long-term SARS-CoV-2 RNA shedding and its temporal association to IgG sero positivity. *Cell Death Discovery* **6**, 138 doi: 10.1038/s41420-020-00375-y
16. **Grigoryan L and Pulendran B** (2020) The Immunology of SARS-CoV-2 Infections and Vaccines. *Seminars in Immunology* **50**, 101422. doi: https://doi.org/10.1016/j.smim.2020.101422
17. **Cohen JI and Burbelo PD** (2020) Reinfection with SARS-CoV-2: Implications for Vaccines. *Clinical Infectious Diseases(CID)*. Dec 18; ciaa1866. doi: 10.1093/cid/ciaa1866. (Online ahead of print)
18. **Leblanc JF *et al.*** (2020) Risk of transmission of severe acute respiratory syndrome coronavirus 2 by transfusion: a literature review. **60**, 3046–3054. doi: https://doi.org/10.1111/trf.160563054.
19. **Chen D *et al.*** (2020) Recurrence of positive SARS-CoV-2 RNA in COVID-19: A case report. *International Journal of Infectious Diseases (IJID)* **93**, 297–299. doi: https://doi.org/10.1016/j.ijid.2020.03.003
20. **Osman A, Al Daajani M and Alsahafi A** (2020) Re-positive coronavirus disease 2019 PCR test: could it be a reinfection? *New Microbes New Infect*.
21. **Dou P *et al.*** (2020) Serial CT features in discharged COVID-19 patients with positive RT-PCR re-test. *European Journal of Radiology* **127**. doi: 10.1016/j.ejrad.2020.109010
22. **Parry J** (2020) Covid-19: Hong Kong scientists report first confirmed case of reinfection. *British Medical Journal (BMJ)* **370**, m3340. doi: 10.1136/bmj.m3340.
23. **Seow J, Graham C and Merrick B** (2020) Longitudinal observation and decline of neutralizing antibody responses in the three months following SARS-CoV-2 infection in humans. *Nature Microbiology* **5**, 1598–1607. doi: 10.1038/s41564-020-00813-8
24. **Tillett RL *et al.*** (2020) Genomic evidence for reinfection with SARS-CoV-2: a case study. *The Lancet Infectious Diseases* **21**, 52–8. doi: https://doi.org/10.1016/S1473-3099(20)307647
25. **Pujadas E *et al.*** (2020) SARS-CoV-2 viral load predicts COVID-19 mortality. *Lancet Respir Med* **8**, e70. doi: 10.1016/s2213-2600(20)30354-4
26. **To KK-W *et al.*** (2020) Coronavirus disease 2019 (COVID-19) re-infection by a phylogenetically distinct severe acute respiratory syndrome coronavirus 2 strain confirmed by whole genome sequencing. *Clinical Infectious Diseasesciaa* 1275. doi: 10.1093/cid/ciaa1275.
27. **Mateus J *et al.*** (2020) Selective and cross-reactive SARS-CoV-2 T cell epitopes in unexposed humans. *Science* **370**, 89–94 doi: 10.1126/science.abd3871.
28. **Weisblum Y *et al.*** (2020) Escape from neutralizing antibodies by SARS-CoV-2 spike protein variants. *Elife* **9**, e61312.
29. **Tomassini S *et al.*** (2020) Setting the criteria for SARS-CoV-2 reinfection-six possible cases. *Journal of Infection* **82**, 282–327. doi: 10.1016/j.jinf.2020.08.011
30. **Qu Y-M *et al.*** (2020) Positive result of Sars-Cov-2 in sputum from a cured patient with COVID-19. *Travel Medicine and Infectious Disease* **34**, 101619. doi: 10.1016/j.tmaid.2020.101619
31. **Lin C *et al.*** (2020) Comparison of throat swabs and sputum specimens for viral nucleic acid detection in 52 cases of novel coronavirus (SARS-Cov-2)-infected pneumonia (COVID-19). *Clinical Chemistry and Laboratory Medicine (CCLM)* **58**, 1089–1094.