
Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Microbial Pathogenesis 161 (2021) 105296

Contents lists available at ScienceDirect

Microbial Pathogenesis

journal homepage: www.elsevier.com/locate/micpath

# SARS-CoV-2 re-infection rate in Iranian COVID-19 cases within one-year follow-up

Mostafa Salehi-Vaziri [a,b], Mohammad Hassan Pouriayevali [a,b], Fatemeh Fotouhi [c], Tahmineh Jalali [a,b,**], Mohammad Banifazl [d], Behrokh Farahmand [c], Mona Sadat Larijani [e], Zahra Ahmadi [a,b], Zahra Fereydouni [a,b], Mahsa Tavakoli [a,b], Afsaneh Karami [f], Sanam Azad-Manjiri [b], Parastoo Yektay Sanati [a], Sarah Dahmardeh [g], Amir Hesam Nemati [a], Marzyie Sajadi [b], Setareh Kashanian [a], Amitis Ramezani [e,*]

[a] COVID-19 National Reference Laboratory, Pasteur Institute of Iran, Tehran, Iran
[b] Department of Arboviruses and Viral Hemorrhagic Fevers (National Reference Laboratory), Pasteur Institute of Iran, Tehran, Iran
[c] Department of Influenza and Other Respiratory Viruses of Pasteur Institute of Iran, Tehran, Iran
[d] Iranian Society for Support of Patients with Infectious Disease, Tehran, Iran
[e] Clinical Research Department, Pasteur Institute of Iran, Tehran, Iran
[f] Department of Infectious Disease, Zanjan University of Medical Sciences, Zanjan, Iran
[g] Vaccination Department, Pasteur Institute of Iran, Tehran, Iran

ARTICLE INFO

*Keywords:*
Re-infection rate
COVID-19
Mutation
SARS-CoV-2, Iran, Reactivation

ABSTRACT

Since the COVID-19 pandemic initiation, the possibility of re-infection has been unclearly present. Although herd immunity has a potential reliance through natural infection, human corona viruses has the ability to subvert immunity and re-infection happens for seasonal corona viruses. Currently, the frequency of SARS-CoV-2 re-infection incidence is not exactly defined. In this study we aimed at determination of SARS-CoV-2 re-infection rate in Iranian population.

In a total of 5696 COVID-19 suspicious individuals, RT-PCR was applied to diagnose the infection. The confirmed patients were followed for 12 months and serology tests were applied to measure the specific antibodies.

Among 1492 confirmed COVID-19 cases, five individuals experienced the subsequent infection. The re-infection/reactivation incidence rate was totally 0.33% after one year of follow-up. The interval ranged from 63 to 156 days. All the cases had viral mutations in the second episode of the infection. All of them were symptomatic cases with moderate severity.

The estimated rate of SARS-CoV-2 in Persian population is therefore rare and natural infection seems to induce good protection against re-infection which clarifies that mass vaccination can hugely affect the society.

## 1. Introduction

Since December 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread globally, infecting over 149 million people across more than 220 countries [1]. There have been some published data which reported cases of possible re-infection s in individuals who had recovered from the prior SARS-CoV-2 episode of infection. Moreover, a growing pool of evidence has highlighted the

* Corresponding author. No:69, Pasteur Ave, Clinical Research Department, Pasteur Institute of Iran, Tehran, 1316943551, Iran.
** Corresponding author. No:69, Pasteur Ave, Department of Arboviruses and Viral Hemorrhagic Fevers, Pasteur Institute of Iran, Tehran, 1316943551, Iran.
*E-mail addresses:* mostafvaziri1985@gmail.com (M. Salehi-Vaziri), m.h.pouria@gmail.com (M.H. Pouriayevali), fotouhi44@yahoo.com (F. Fotouhi), jalalitahmineh@gmail.com (T. Jalali), mohammad.banifazl@aol.com (M. Banifazl), b_farahmand@pasteur.ac.ir (B. Farahmand), mona.sadat@gmail.com (M. Sadat Larijani), zahraahmadii1995@gmail.com (Z. Ahmadi), zahrafereydouni70@gmail.com (Z. Fereydouni), mahsa.tavakolirad@gmail.com (M. Tavakoli), dr.akarami@yahoo.com (A. Karami), s.a_92@yahoo.com (S. Azad-Manjiri), p.yekta.oloom@gmail.com (P. Yektay Sanati), sarahdahmardeh@gmail.com (S. Dahmardeh), hesam.nemati@live.com (A.H. Nemati), marziiie.97@gmail.com (M. Sajadi), setareh.kashanian76@gmail.com (S. Kashanian), amitisramezani@hotmail.com (A. Ramezani).

https://doi.org/10.1016/j.micpath.2021.105296
Received 19 June 2021; Received in revised form 13 November 2021; Accepted 14 November 2021
Available online 18 November 2021

recurrent infection possibility within a few days post recovery from coronavirus disease 2019 (COVID-19) [2–5]. Therefore, the challenges in determining true re-infections whereas persistent viral shedding are still discussed. Therefore, additional lines of assessment to support a re-infection diagnosis have been highlighted including sequencing of viral genes [2,4,5].

A true diagnosis of SARS-CoV-2 re-infection must be established when complete viral clearance is achieved after the first episode of infection. Moreover, sufficient time is needed to allow for mounted immune responses [6]. The studies which focus on characterizing COVID-19-related immunity have shown that binding and neutralizing antibodies developed in response to SARS-CoV-2 infection and could provide protection up to six months after the onset of symptoms. Antibody titers establishment as a correlated factor with protection should be defined as a protective element which would be extremely critical for both patient management and public health considerations [7,8]. In fact, the importance of re-infection determination in a community can reflect the quarantine policy and vaccine development strategies. Although the cause of SARS-CoV-2 re-infection/reactivation has not been fully characterized, some factors were found to be associated with its possibility including age and underlying diseases [9]. What is more, a study on a large population has revealed that individuals who once experienced COVID-19, had lower re-infection and symptomatic infection risks of 84% and 93% respectively within 7 months of follow-up [10].

The rates of protection against subsequent infections of SARS-CoV-2 is still little known although some studies suggest that SARS-CoV-2 re-infection occurs in less than 1% of those who previously were positive for SARS-CoV-2 [11,12]. Nevertheless, most of the findings are based on several single or small case studies. Therefore, determination of the re-infection rate in large population can contribute to a better understanding. In other words, more epidemiological case definition of SARSCoV-2 re-infection is crucial to strengthen community surveillance. In this study, determination of COVID-19 re-infection rate among Iranian population was investigated.

## 2. Materials and methods

In this study, 5696 COVID-19 suspected cases were enrolled and studied at COVID-19 National Reference laboratory at Pasteur Institute of Iran. The SARS-CoV-2 diagnosis and treatment was performed according to the standard flowchart in Iran (3rd edition) [13]. Viral RNA extraction was done by a QIAcube HT system with a QIAamp 96 Virus QIAcube HT Kit, according to the manufacturer's instructions. Real Time Reverse-transcription PCR assay was applied using 2019-nCoV Nucleic Acid Diagnostic kit (Sansure biotech, Changsha, China), in accordance to the manufacturer's protocol. SARS-CoV-2 specific antibodies were tracked by IgM Capture kit (cat no: PT-CoV-2-IgM Cap-96 [sensitivity 70%, specificity: 75%] Pishtazteb, Iran) and IgG kit (cat no: PT-CoV-19 IgG-96, [sensitivity 78%, specificity: 91%] Pishtazteb, Iran) according to the provided protocol. The full recovery of COVID-19 was described as two RT-PCR tests with negative results with 24 h of interval. This study was approved by the ethics committee of Pasteur Institute of Iran (approval ID: IR.PII.REC.1399.064).

## 3. Results

During the one-year period of this investigate and admission of 5696 COVID-19 suspected subjects, 1492 cases were confirmed. The confirmed patients were followed up for one year. Symptomatic cases were considered for molecular and serological tests for COVID-19. Considering both re-infection and viral reactivation, the subsequent infection was recorded for five individuals, 0.33%, among all from whom four were males and one was female ranging from 32 to 54 years old. The interval between the two episodes ranged from 63 to 156 days. The sequencing results showed different mutation patterns between first and second episodes in two cases indicating the occurrence of re-infection (No.4, 5) (Table 1). By the way, in two subjects (No.1, 3) we had no access to first sample but D614G mutation was detected at the second time. According to the time of first infection, the predominant circulating virus was D614 and few months later, G614 type became dominant in our country. Therefore, we considered these cases as possibly re-infected ones (Table 1).

One case sequencing revealed L139L non-synonymous mutation in both episodes that was assessed as a SARS-CoV-2 reactivated case.

Moreover, four cases were found IgG positive in the re-infection phase except one whose full viral sequencing showed 6 new mutations in the second episode in comparison with the first incidence [14,15].

All of them were symptomatic cases with moderate severity except one case who presented with mild infection in first episode and moderate infection with lung involvement in second time (case No.5). Therefore, re-infections mostly were well-tolerated and no more symptomatic than first infections.

Additionally, we had four other symptomatic cases compatible with COVID-19 presentations, and SARS-CoV-2 RT-PCR tests positive but probably due to the low quality of samples and high cycle threshold, these cases were not confirmed and documented by viral genome sequencing as re-infection individuals.

## 4. Discussion

The possibility of SARS-CoV-2 re-infection or reactivation can significantly reflect clinical implications and also vaccination strategy. In the present study, we assessed the frequency of SARS-CoV-2 re-infection/reactivation in Iranian population according to a long term follow-up and the previous studies on re-infection case reports. This study showed that the documented subsequent infection in our population is 0.33% which can be defined as a rare incidence. All of them were symptomatic cases with moderate severity. Our results suggested that re-infections are mostly well tolerated and no more symptomatic than first infections.

In a study which was done on 3249 participants from the U.S, 189 individuals were seropositive from whom 10% had at least one SARS-CoV-2 positive PCR test during the 6-week follow-up. In contrast, 48% of the seronegative individuals tested positive and the incidence rate ratio was assessed 0.18. Moreover, among the seropositive cases with lower baseline of spike protein IgG titers, infection rate was more than in those who had higher baseline of SIgG titers [16].

SARS-CoV-2 re-infection investigate in India revealed the rate of 4.5% (58 out of 1300 cases). Furthermore, the vast majority of the participants was asymptomatic and showed higher Ct value during the first infection [17].

The other survey which was done in Denmark showed that 0.65% of the confirmed COVID-19 cases in the first incidence reached positive PCR again after the follow-up. In comparison those who were PCR negative in the first step of the study, 3.27% developed the infection during the follow-up. Therefore, they found that protection against re-infection was 80.5%. Moreover, age was found an important factor for repeated infection as protection was 47.1% among those older than 65 years [18].

The other study by Abu-Raddad et al. on 43,044 positive cases who were followed for about 4 months, 0.7% individuals had a second positive PCR two weeks after the positive serology test. From this population, 41% had epidemiological lines of evidence for the re-infection. The final sequencing assessment recorded 0.1% re-infection rate in the investigated population in Qatar [19]. Most of Re-infection cases were mildly symptomatic whereas 1 severe and 2 moderate subjects. These results suggested that most of re-infections had-well tolerated condition.

Pilz et al. reported low re-infection rate of SARS-CoV-2 in Austria. These findings showed that natural infection might induce strong protection against re-infection which is comparable with vaccine efficacious in Austria [20].

**Table 1**
Covid-19 re-infected/reactivated cases characteristics.

| *Cases* | No.1 | No.2 | No.3 | No.4 | No.5 |
|---|---|---|---|---|---|
| *Gender* | Female | Male | Male | Male | Male |
| *Age* | 32 | 54 | 42 | 42 | 32 |
| *Underlying Disease* | - | - | - | - | - |
| *First Infection* | 2020/04/20 | 2020/04/04 | 2020/03/10 | 2020/07/4 | 2020/11/18 |
| *IgG* | - | + | N/A | - | - |
| *Second Infection* | 2020/07/17 | 2020/08/22 | 2020/07/04 | 2020/11/9 | 2021//04/11 |
| *N CT*[a] | 17 | 29 | 36 | 18 | 12 |
| *ORF CT* | 18 | 30 | 37 | 17 | 14 |
| *IgG* | + | + | + | - | + |
| *Infection interval* | 63 days | 156 days | 111 days | 128 days | 144 days |
| | Partial or whole genome sequencing | Partial, | Partial, | Partial, | Whole genome | Partial |
| *SARS-COV-2 Mutations* | *First episode* | N/A | L139L non-synonymous mutation | N/A | NC. 241C>T<br>F 106 F<br>T 2685 P<br>P 323 L<br>L 280 L (non-synonymous)<br>I 210 del<br>D 294 D<br>D 614 G<br>Q 57 H<br>Y 71 Y (non-synonymous<br>S 194 L | D 138 Y<br>S 477 N |
| | *Second episode* | D614G | L139L non-synonymous mutation | D614G | NC. 203C>T<br>NC. 241C>T<br>E 37 D<br>F 106 F (non-synonymous)<br>T 2007 I<br>P 323 L<br>R 52 K<br>L 280 L (non-synonymous)<br>I 210 del<br>D 294 D<br>D 614 G<br>Q 57 H<br>Y 71 Y<br>F 110 F (non-synonymous)<br>S 194 L<br>Y 268 Y (non-synonymous) | HV 69/70 del<br>Y 144 DEL<br>N 501 Y<br>A 570 D |
| *Probable Diagnosis* | Re-infection | Reactivation | Re-infection | Re-infection | Re-infection |

N/A: *Not applicable*.
[a] CT: Cycle threshold.

Our study is compatible with other investigations regarding scarce rate of SARS-CoV-2 re-infections. During the COVID-19 infection, the induced antibodies by initial infection can be largely protective. However, they might not guarantee preventive SARS-CoV-2 further immunity against subsequent infection. These findings from different population can be practical for mass vaccination strategies. Although SARS-CoV-2 re-infection has been still a rare phenomenon, the epidemiological definition of re-infection is required for surveillance establishment and this study contributes to such an essential goal.

### *4.1. Limitations*

A limitation of the current study is that the few cases were not confirmed and recorded as re-infected individuals by viral genome sequencing due to low sample quality and high cycle threshold.

## Funding

This study was financially supported by Pasteur Institute of Iran (Grant number: 1893).

## CRediT authorship contribution statement

Mostafa Salehi-Vaziri, Mohammad Hassan Pouriayevali, Tahmineh Jalali: Supervision, Validation. Fatemeh Fotouhi, Behrokh Farahmand: Methodology. Mohammad Banifazl, Afsaneh Karami, Sarah Dahmardeh: Patients' visits. Zahra Ahmadi, Zahra Fereydouni, Mahsa, Tavakoli, Sanam Azad-Manjiri, Parastoo Yektay Sanati, Amir Hesam Nemati, Marzyie Sajadi, Setareh Kashanian: Methodology. Mona Sadat Larijani: Data curation, Formal analysis, Writing-Original draft preparation. Amitis Ramezani: Conceptualization, Project administration, Writing-Review & Editing. All authors read and approved the manuscript.

## Declaration of competing interest

Authors mention that there is no conflict of interest in this study.

## Acknowledgment

We appreciate the Pasteur Institute of Iran for the financial support of this study.

## References

[1] WHO Coronavirus Disease (COVID-19) Dashboard, World health Organization (WHO) [Internet],[cited 2021/04/30]. Available from: https://covid19.who.int/, 2021.
[2] Re-infection with SARS-CoV-2: considerations for public health response [Internet]. Stockholm: ECDC. [cited 2021/04/30]. Available from: https://www.ecdc.europa.eu/en/publications-data/threat-assessment-brief-re-infection -sars -cov-2.
[3] Iwasaki A. What Re-infection S Mean for COVID-19. The Lancet Infectious Diseases..
[4] Tillett RL, Sevinsky JR, Hartley PD, et al. Genomic Evidence for Re-infection with SARS-CoV-2: a Case Study. The Lancet Infectious Diseases..

[5] J. Parry, Covid-19: Hong Kong scientists report first confirmed case of re-infection, BMJ 370 (2020) m3340.
[6] Control ECfDPa, Re-infection with SARS-CoV-2: Implementation of a Surveillance Case Definition within the EU/EEA, 2021, 8 April 2021.
[7] M. Sofian, A.A. Velayati, M. Banifazl, et al., SARS-CoV-2, a virus with many faces: a series of cases with prolonged persistence of COVID-19 symptoms, Wien Med. Wochenschr. 171 (2020) 3–6.
[8] F. Fotouhi, M. Salehi-Vaziri, B. Farahmand, et al., Prolonged viral shedding and antibody persistence in patients with COVID-19, Microb. Infect. (2021) 104810.
[9] [Internet], Researchers Identify Risk Factors for SARS-CoV-2 Re-infection, News Medical Life Science, 2021. Available from: https://www.news-medical.net/news/20210416/Researchers-identify-risk-factors-for-SARS-CoV-2-re-infection .aspx.
[10] V.J. Hall, S. Foulkes, A. Charlett, et al., SARS-CoV-2 infection rates of antibody-positive compared with antibody-negative health-care workers in England: a large, multicentre, prospective cohort study (SIREN), Lancet 397 (10283) (2021) 1459–1469.
[11] G.A. Poland, I.G. Ovsyannikova, R.B. Kennedy, SARS-CoV-2 immunity: review and applications to phase 3 vaccine candidates, Lancet 396 (10262) (2020) 1595–1606.
[12] V. Hall, S. Foulkes, A. Charlett, et al., Do Antibody Positive Healthcare Workers Have Lower SARS-CoV-2 Infection Rates than Antibody Negative Healthcare Workers? Large Multi-Centre Prospective Cohort Study (The SIREN Study), England: June to November 2020. medRxiv, 2021, 2021.01.13.21249642.
[13] [Internet], The COVID-19 Diagnosis and Treatment Flowchart in Iran, third ed., 2020 [cited 18 March, 2020]. Available from: http://treatment.sbmu.ac.ir/index.jsp?siteid=62&fkeyid=&siteid=62&pageid=63989.
[14] T.J. Mostafa Salehi-Vaziri, Behrokh Farahmand, Fatemeh Fotouhi, Mohammad Banifazl, Mohammad Hassan Pouriayevali, Mona Sadat Larijani, Afzali Neda, Amitis Ramezani, Clinical Characteristics of SARS-CoV-2 by Re-infection vs. Reactivation: A Case Series from Iran, European Journal of Clinical Microbiology & Infectious Diseases, 2021.
[15] M. Salehi-Vaziri, M.D. Omrani, M.H. Pouriayevali, et al., SARS-CoV-2 presented moderately during two episodes of the infection with lack of antibody responses, Virus Res. 299 (2021) 198421.
[16] AG Letizia, Y Ge, S Vangeti, et al., SARS-CoV-2 seropositivity and subsequent infection risk in healthy young adults: a prospective cohort study, Lancet. Resp. Med. 9 (7) (2021) 712–720.
[17] A. Mukherjee, T. Anand, A. Agarwal, et al., SARS-CoV-2 re-infection: development of an epidemiological definition from India, Epidemiol. Infect. 149 (2021) e82.
[18] C.H. Hansen, D. Michlmayr, S.M. Gubbels, K. Mølbak, S. Ethelberg, Assessment of protection against re-infection with SARS-CoV-2 among 4 million PCR-tested individuals in Denmark in 2020: a population-level observational study, Lancet 397 (10280) (2021) 1204–1212.
[19] Laith J. Abu-Raddad, Hiam Chemaitelly, Peter Coyle, et al., et al., SARS-CoV-2 antibody-positivity protects against reinfection for at least seven months with 95% efficacy, E Clin. Med. 35 (100861) (2021), https://doi.org/10.1016/j.eclinm.2021.100861.
[20] S. Pilz, A. Chakeri, , Ioannidis 3, J. Lukas Richter, V. Theiler-Schwetz, C. Trummer, SARS-CoV-2 re-infection risk in Austria, Eur. J. Clin. Invest. 51 (4) (2021), e13520.