COVID-19 Reinfection in Shahroud, Iran; A follow up Study

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Summary

Although many people became infected and recovered during the Covid-19 epidemic, the immunity duration and reinfection in recovered patients has 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 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 March 20, 2020 to November 20, 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 reinfection 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 to 234 days). The risk of reinfection between male and females was not statistically different (1.98 per 1,000 women and 2.96 per 1,000 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 emphasized especially in health care personnel who are more exposed to the virus.

Key words: COVID-19, incidence, reinfection, SARS-COV
Introduction

The first case of Covid-19 was discovered in Wuhan, China in December 2019 and soon spread around the world [1]. To date (January 28, 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 to 22 days for complete removal of virus from patient’s body [10 -12]. 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, 7, 13]. Due to various duration of the immune response in 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 hours apart considered as 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 [3-5] Korea [6] USA [7] Brazil [8] about 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, so our aim in this study was to evaluate the rate of re-infection over a 9 month period after onset of Covid-19 epidemic in Shahroud, a city located in northeastern Iran.
Methods

The Shahroud is located in northeastern Iran with a population of 285,000. [9] Covid-19 patients are admitted to a referral hospital in the area, and there are 96 health centers 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 reinfection 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 which 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 March 20, 2020 to November 20, 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 hospitalized 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 hospitalizations 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 one-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 from 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 to 234 days). Out of these 10 definitive re-infected patients, 4 were female and 6 were male (Table 1). Four of them were admitted to the intensive care unit (ICU) both in primary infection and re-infection period and 4 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. One had both history of bone marrow cancer and chemotherapy due to leukemia and also heart disease.
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 are 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 [5, 7, 19, 20]. But the remarkable point was the younger age range (between 21-60 years) that other studies have reported in re-infection of Covid-19 [8, 21, 22]. Due to the fact that seroconversion is observed in most patients with SARS-COV-2, however, the titer of binding and neutralizing 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 neutralizing antibody titers, and that antibody levels are still detectable 2 to 3 months after primary symptoms, while those who were asymptomatic or had mild symptoms, had lower antibody titers, and in less than two 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 titer of antibodies needed to neutralize the virus to prevent re-infection must be determined [4]. 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 [12].

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, 4 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 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 analyzes 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 neutralizing antibodies or at the response site of CD4 cells [26-28] and so these protein changes may make the virus less exposed to the neutralizing 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 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 centers 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 emphasized especially in health care personnel who are more exposed to the virus.

**Declaration of competing interests**

The authors declare that there is no conflict of interest

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**Availability of data and materials**

The datasets used during the current study are available from the corresponding author on reasonable request
Table 1. Characteristics of 10 covid-19 reinfection patients

| Sample Date(1) | Sex | Age (year) | Duration of Hospitalization (Days) | Discharge Date(1) | Sample Date(2) | Duration of Hospitalization (Days) | 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 Jun          | Female | 90       | 21                                 | 25 Jun            | 10 October     | 12                                 | 22 October        | 130                                        | Survive |
| 6 July         | Female | 13      | Outpatient                        | N/A               | 7 November     | Outpatient                         | N/A               | 124                                        | Survive |
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