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SARS-CoV-2 presented moderately during two episodes of the infection with lack of antibody responses

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ABSTRACT

The world has gone through the critical phase of SARS-CoV-2 crisis caused by the new variants of the virus. The globally concerted effort to characterize viral genomic mutations across different clades has revealed several changes in the coding and also non-coding regions which might lead to a violent presentation or re-infection occurrence.

Here, we studied a COVID-19 subject who represented the symptoms following the full recovery of the first infection. COVID-19 specific IgM and IgG were evaluated in both steps. The viral samples from oropharyngeal/nasopharyngeal were subjected to RT-PCR and full sequencing was done in both incidences. The sequencing data was fully investigated with the reference sequence of SARS-CoV-2 and the changes were detected.

The obtained data is in favor of re-infection with 128 days of interval. SARS-CoV-2 presented more severely in the second episode of the disease and the specific antibodies against COVID-19 were not detectable. Both infections were caused by the same clade 20G, however, the mutation rates were higher in the second incidence including 10 nucleotide substitutions which had rarely been reported before. In the present study, the nucleotide mutations in various regions of the viral genome have been presented. The re-infection could have significant effect on clinical implications as well as vaccination.

1. Introduction

Although SARS-CoV-2 usually results in a detectable immune response, the possibility of re-infection in previously infected individuals is still a crucial concern. There have been lines of evidence from available studies in which a healthy immune response normally develops in COVID-19 infected subjects (Sewell et al., 2020; Thomas, 2020; Udugama et al., 2020; Zhou and Zhao, 2020). Therefore, the immune response should be able to block a second infection according to the generated antibodies and memory cells against the virus. The recovered patients from the infection are then supposed to be protected from another possible infection at least for a definitive time, however, the duration of this period has not been well identified, yet (Catanzaro et al., 2020; Siracusano et al., 2020). The knowledge of the exact protective mechanism provided against SARS-CoV-2 needs to be grown as well as the required levels of humoral or cellular responses. Age, the immunity system level and probable virus mutations are among the factors which make the re-infection possible (Sofian et al., 2020; To et al., 2020a).

Some studies indicate that SARS-CoV-2 with mild presentation leads to weaker immune activity and subsequently short protection rather than severe infection (Garcia, 2020; Shi et al., 2020). SARS-CoV-2

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infected individuals mostly reach an antibody response during 10–21 days post infection. Nevertheless, minority of cases do not develop detectable IgM and/or IgG (ORBA et al., 2020; Woelfel et al., 2020; Zhao et al., 2020). Some investigated cases generated antibodies for about six months after the infection. (Fotouhi et al., 2021; Long et al., 2020; To et al., 2020b; Wu et al., 2020). The reported cases from different geographic places has brought the concern that re-infection of SARS-CoV-2 might affect the efficacy of emerging vaccines (Nachmias et al., 2020; Parry, 2020; Saplakoglu, 2020).

Here, we report the analytic data of a re-infected SARS-CoV-2 by genetic evaluation in both episodes of the infection.

2. Materials and methods

2.1. Case presentation

2.1.1. First episode

On 2020/07/04, a 42-year-old man with cough, headache and severe diarrhea (lasted for 4 days), with a history of close contact with a confirmed COVID-19 case but no underlying diseases was referred to the COVID-19 National Reference Laboratory at Pasteur Institute of Iran.

The oropharyngeal/nasopharyngeal swabs were obtained and viral RNA was extracted using a QIAcube HT system with a QIAamp 96 Virus QIAcube HT Kit, according to the manufacturer’s protocol. Real Time Reverse-transcription PCR (Real Time RT-PCR) assay was done by 2019-nCoV Nucleic Acid Diagnostic kit (Sansuno biotech, Changsha, China), according to the manufacturer’s guide. Sera was collected from the blood sample on 29th July, 2020 to detect SARS-CoV-2 antibodies, SARS-CoV-2 IgM Capture kit (cat no: PT-CoV-2-IgM Cap-96 [sensitivity 70 %, specificity: 75 %]) Pishtazteb, Iran) and SARS-CoV-2 IgG kit (cat no: PT-CoV-19 IgG-96, [sensitivity 78 %, specificity: 91 %]) Pishtazteb, Iran) were employed according to the provided protocol. The ELISA test was rechecked at the same day.

2.1.2. Second episode

On 2020/11/9, the patient sought medical treatment for body pain, shortness of breath, headache and anosmia following exposure to a confirmed patient at home. The symptoms initiated more severe than the first episode (Table 1). The same COVID-19 diagnostic assays of the first episode were employed to confirm the SARS-CoV-2 infection on 2020/11/9 and antibody assessment was also done on 2020/11/18.

3. Results

Real Time RT-PCR results of the first incidence confirmed SARS-CoV-2 infection (N gene Ct values: 16, ORF1ab gene Ct values: 19). No antibody response was detected in patient’s serum sample by ELISA.

Following a two-week isolation period, two consecutive oropharyngeal/nasopharyngeal swabs were taken from the case in a 24 -h period and subjected to Real Time RT-PCR. Both Real Time RT-PCR tests resulted negative.

Real Time RT-PCR results of the second episode indicated SARS-CoV-2 infection (N gene Ct values: 16, ORF1ab gene Ct values: 17), but no antibody response was detected by ELISA.

Considering the two confirmed SARS-CoV-2 infection with an interval of 128 days, full genome sequencing of the viruses detected in both episodes was performed using 40 pairs of primers (Lu et al., 2020) and RT-PCR one step RT-PCR Kit (biotecnabbit, Germany) as described in our previous study (Mostafa Salehi-Vaziri et al., 2021). RT-PCR products were visualized by gel electrophoresis and sequenced bidirectionally by the Applied Biosystems 3500xl GeneticAnalyzer. Raw sequencing data was trimmed and consensus sequence was made using the CLC Main Workbench 5.5 package (CLC bio, Denmark). The mutations were determined according to SARS-CoV-2 Sequence Resources (NC_045512).

The whole genome of virus from the first and second infections were successfully sequenced and submitted to the GISAID databank the GISAID (https://www.gisaid.org/) under the accession numbers EPI_ISL_847826 and EPI_ISL_847827, respectively. Phylogenetic analysis was performed using NextStrain online tools (https://nextstrain.org/).

Although the first (EPI_ISL_847826) and second (EPI_ISL_847827) episodes related viruses were found to be a member of the clade 20 G (Fig. 1), the sequencing data showed different mutation pattern in each sample (Table 2). Genomic sequence analysis of the first virus identified 11 mutated positions compared to the reference genome. The second virus, had six additional mutations from which 10 mutations were similar to the first virus including c. 241 C > T at 5’UTR, 3037 C > T in ORF1ab, NSP3, 14408 C > T in ORF1ab, NSP12, 18877 C > T in ORF1ab, NSP14, 22191-3 DEL, 2244 C > T and 23403 A > G in Spike, 25563 G > T in NS3, 26735 C > T in Matrix and 28854 C > T in Nucleocapsid (Table 2).

Sequence data showed that the first collected specimen was a member of clade 20G (Fig. 1) and genomic sequence analysis identified 11 mutation positions compared to the reference genome (Table 2). The second specimen, collected was belonged to the same clade, presenting six additional mutations in comparison with the first incidence (Table 2), among the total mutations, six were synonymous. What is more, 10 nucleotide substitutions had not been reported before (according to the entropy index at the date of the search).

4. Discussion

The genetic differences occurring in SARS-CoV-2 strains could be associated with their geographical distributions which make this virus capable of adapting swiftly to the wide environments. (Islam et al., 2020). Accumulated mutations within the genome is a primary force in viral evolution during an endemic era. The acquisitive features usually lead to severe infectivity, altered virulence and better transmissibility besides antigenic shifting which facilitates host immunity escape. This also might compromise the vaccines efficacy and antiviral drugs (Sadat et al., 2021; Sarkar et al., 2021).

The data analysis in this study supports an instance of probable SARS-CoV-2 re-infection. The reported case experienced 6 more mutations compared to the first occurrence. His illness presented more severely in the re-infection phase which might be associated with the mutated genes. However, in none of the incidences SARS-CoV-2 specific antibody was detected.

To confirm that the reported case experienced a re-infection, the viruses of both incidences were analyzed by sequencing and six different mutations were found in the second excluded virus. Hence, we assumed that these viruses were different a re-infection was probable. From another point of view, it was probable that an ongoing evolution of the initial virus within the patient happened. Nevertheless, this case developed the second infection after facing a confirmed infected relative who caused the whole family COVID-19 infection. Therefore, it seems the second source of the virus was new and this highlights the re-

### Table 1

| Features                      | First infection | Second infection |
|-------------------------------|-----------------|------------------|
| Underlying disease            | –               | –                |
| PCR date                      | 4th, Jul, 2020  | 9th, Nov, 2020   |
| The interval to re-infection  | 128 days        |                  |
| Ct (N)                        | 18              | 16               |
| Ct (ORF1ab)                   | 19              | 17               |
| IgM                           | Neg             | Neg              |
| IgG                           | Neg             | Neg              |
| History of exposure           | Confirmed case at workplace | Confirmed case in family |
| Symptoms                      | Cough, headache, severe diarrhea | body pain, shortness of breath, headache and anosmia |
In a study by Petersen et al. on 2547 cases with previously confirmed SARS-CoV-2 infection, 160 persons, 6.3%, were found seronegative. Moreover, the seronegative ranged from 0% among 79 persons previously hospitalized to 11.0% among 308 persons with asymptomatic infections. The concluded that lack of IgG was seen in 1 out of 16 persons and this antibody absence varied independently from immunosuppressive drug therapy, illness severity or race/ethnicity (Petersen et al., 2020).

In the other investigation on 156 frontline U.S. health care staff who were followed up for 60 days, 93.6% had a decline in antibody levels and 28.2% had complete seroreversion. What is more, 64.9% of those with low antibody levels seroreverted whereas 7.1% of those did with high IgG titers. Similar to our findings, these results indicate that a substantial population of SARS-CoV-2 infected individuals might have negative serologic responses in the months following infection (Self et al., 2020).

Several important implications could be provided from the findings. The seroconversion might not happen in some individuals even with healthy immunity system which may result in re-infection. Furthermore, application of serologic assessment results to detect previous SARS-CoV-2 infection is under question according to these results. It can be assumed that humoral immunity to a primary infection caused by a novel virus might not be durable or strong. Although the developed memory B-cell and T-cell responses caused by the first incidence are supposed to act which could reduce the illness severity in the re-infection phase, the patient in this study felt worse at this stage. In addition to all discussed above, the possible evolution of the initial virus in the patient needed more investigation on different excluded samples in various times and we could not roll out this issue. Therefore, in futuristic investigations, this should be aimed to distinguish the real re-infection from virus evolution. In addition to this study, other recent data have mentioned certain mutations at amino acid position in both structural and non-structural proteins of SARS-CoV-2. These results indicate SARS-CoV-2 is probably undergoing adaptive evolution as the consequence of consecutive mutations in S and also non-structural proteins.
proteins. Therefore, a concise mutations surveillance is essentially needed.

5. Conclusions
SARS-CoV-2 like other RNA viruses has the potency of a high mutation rate and hence, is prone to bring drugs resistance and vaccine failure. In the present study, the nucleotide mutations in various regions of the viral genome have been presented. According to high rate of the mutations and lack of antibody responses, this case has been supposed to be re-infected who presented moderate COVID-19 in both episodes.

Authors’ statement
Mostafa Salehi-Vaziri, conducted the study and contributed to the writing; Mir Davood Omrani, supervised the study and provided technical support; Mohammad Hassan Pouriaievali, conducted the genetics evaluation and provided technical advice; Fatemeh Fotouhi, designed the laboratory steps and supervised the study; Mohammad Banifazl, handled the patients and supervised the project; Behrokh Farahmand, was involved in the technical procedure and supervision; Mona Sadat Larijani did the related research and wrote the primary manuscript; Zahra Ahmadi, Zahra Fereydouni and Mahsa Tavakoli conducted the laboratory tests; Tahmineh Jalali, supervised the project and the methodology; Amitis Ramezani as the main corresponding author, supervised the project, provided technical support and finalized the paper.

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Ethics
The study was approved by Pasteur Institute of Iran Ethical committee under IR.PILREC.1399.064 ethical code.

Declaration of Competing Interest
The authors report no declarations of interest.

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