Detection of SARS CoV-2 reinfections by rapid inexpensive techniques

Sherko Subhan Niranji (sherko.subhan@garmian.edu.krd)  
University of Garmian  https://orcid.org/0000-0001-9210-0129

Sirwan M.A. Al-Jaf  
University of Garmian  https://orcid.org/0000-0003-0203-1022

Research Article

Keywords: Reinfections, SARS CoV-2, B.1.1.7, Iraq, Method

DOI: https://doi.org/10.21203/rs.3.rs-558215/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.  
Read Full License
Abstract

Reinfections of SARS CoV-2 are rare in the world and it is difficult to be confirmed whether it is a reinfection or persistent infection. The most prominent factors used for differentiating the reinfections from persistent infections are whole genome sequencings and phylogenetic analyses that require times and funds, which may not be feasible in most developing countries. We previously developed rapid economical methods to identify both D614G and N501Y mutations in clinical samples using rRT PCR probes and endpoint PCR specific primers. Current study has found an immunocompetent patient with a SARS CoV-2 N501Y reinfection without comorbidities. The results suggested that the initial infection was due to a variant contained only D614G mutation while the reinfection was potentially as result of the UK variant contained three mutations confirmed by DNA sequences, including D614G, N501Y and A570D mutations. Seven cases of reinfections were also confirmed by these methods suggested that these techniques will support rapid detection of SARS CoV-2 reinfections in developing countries where sequencing tools are unavailable.

Introduction

SARS CoV-2 reinfections are rare and happen as a result of declining antibodies in convalescent people. However, recent study found that a reinfection occurs in an immunocompetent person who had neutralizing antibodies produced from the initial infections (1). The infections generally persist in immunosuppressed patients but the reinfections occur in immunocompetent individuals in regions where different variants are circulating (2); this is often confusing to differentiate between reinfections and persistent infections. The reinfections can be differentiated from persistent infections by identifications of the viral variants in the clinical samples taken from both first and second infections. Viral variants are usually identified by phylogenetic analysis of the viral whole genome sequences which are difficult to obtain as quickly as possible for both initial and second infections (3). In other words, confirmations of reinfections have been under investigations. CDC recommends several criteria for suspecting SARS CoV-2 reinfections including collecting respiratory samples daily for 7 days, and serum samples in different times points in days 3, 7, 14, 21, 60 (4). Therefore, alternative, rapid, molecular biological methods can also be exploited for variant identifications using rRT PCR probes, particularly when whole genome sequencings are not available in poor or developing countries. Several methods have recently been developed (5–9). These methods are more inexpensive and feasible than whole genome sequencings.

Before emergence of SARS CoV-2 variants of concerns such as B.1.1.7, B.1.351, P1 and B.1.617, reinfections were seldomly reported in the world. For example, a reinfection with SARS CoV-2 was first reported in a person from Hong Kong migrated to Europe that was confirmed to be caused by a different lineage of the virus (10). Later on, several cases of re-infections have been reported worldwide (2). However, the variants of most of these reinfections were not confirmed by genomic sequencings; this makes reporting reinfections controversial and confusing with persistent infections (11). In other words,
the majority of the global SARS CoV-2 reinfections may have been a persistent infection not even reinfections (12). This highlights the importance of the reinfection confirmation and persistent infection exclusions by using either whole genome sequencings or rapid techniques to identify the virus's variants.

SARS CoV-2 has three major variants of concerns (VOC), which have been known to influence on the transmissibility, infectivity and fatality of the virus, including B.1.1.7 (UK variant) (13, 14), B.1.351 (South African variant), P.1 (Brazil variant) and B.1.617 (Indian variant). Up to our best knowledge, there are few case reports of reinfections in Iraq (15, 16). Nonetheless, no studies have confirmed reinfection using DNA sequencings, particularly with the UK variant and few reports in the world. This was possibly due to difficulties in comparing the whole genome sequences between the initial infections and reinfections. In this study we aim to investigate reinfections in a person reported in Kalar town, Sulaymaniyah province, Kurdistan regional government of Iraq, where sequencing facilities are hardly obtained. We also aim to apply the previous rapid methods to confirm reinfections with variants carrying N501Y mutations that have occurred in the region.

**Materials And Methods**

Firstly, a person was presented to Coronavirus Research and Identification lab, University of Garmian, Kurdistan Region, Iraq. He agreed to fill a consent form as a participant of this study, which was approved by an ethical committee at the Department of Biology, University of Garmian that follows the rules adhered to the Declaration of Helsinki for human and animal research. He was 42 years old, his body weight was 70, height= 170 cm. Nasopharyngeal swab was taken in viral transport medium (VTM) on 7th October 2020. The initial infection was tested for diagnosis as SARS CoV-2 using a coronavirus real time RT-PCR kit (MutaPLEX® immundiagnostik, Germany). Blood tests were performed to observe complete blood counts (CBC), ferritin, D dimer, LDH, CRP and ESR. The patient had no comorbidities. The reinfection was diagnosed on 23 March 2021 (after 5.5 months) using same protocol as the initial infection.

Identifications of SARS CoV-2 N501Y and D614G mutations were performed for both initial infection and the reinfection, using rapid molecular biological methods as previously developed (5, 6). These were confirmed by DNA sequencings. An ELISA test for SARS CoV 2 IgG and IgM (Ideal Tashkhis Atieh co. Iran) was performed a day post-re-infection. The rapid molecular methods were also applied to 7 persons suspected with either reinfections or persistent infections. They agreed to fill a consent forms and approved by the ethical committee as previously mentioned.

**Results**

**Initial infection**

The clinical features were as follows: severe sore throat with excessive cough for two weeks that was initially dry but wet and purulent at the end. Sneezing, malaise, fatigue, diarrhoea were also present. Oxygenation saturations was normal as indicated by normal
SPO2 more than 96%. The results of the rapid methods showed that the initial infection resulted from a SARS CoV-2 variant, which have had N501 wildtype and D614G mutant. The viral load was Ct value= 17.14. The sequencing result also confirmed amino acids N501 wildtype, A570 wildtype, D614G mutant in the initial variant (MW897351). Treatment was Vitamin D, Zinc, Vitamin C, Paracetamol and Azithromycin for 10 days. PCR result was negative after 2 weeks. Another PCR test was performed 4 months later.

**Reinfection**

The clinical features were as follows: moderate sore throat with mild coughs for 10 days. Dry mouth was much more than the initial infection. Malaise and fatigue were less prominent than the previous infection, and the patient had no diarrhoea. He had normal SPO2. The rapid methods and sequencing result (MW897356) found two amino acid changes N501Y, and A570D, in addition to the D614G which was also present in the initial infection. Presence of Y510, D570 and G614 mutations in the reinfection suggested the UK variant. Other parameters are as follows: Ct value= 17.9, normal CBC, ferritin, D dimer, LDH and ESR, but CRP was 15 mg/dl (normal CRP titer less than 5 mg/dl). Treatment was Vitamin D, Zinc, Vitamin C, Paracetamol and Azithromycin for 7 days. The patient recovered with negative PCR after 2 weeks with no clinical signs. Both IgG and IgM were considered as negative results with very low titres, 0.28 and 0.182, respectively.

**Application of the rapid molecular biological methods on other re-infections**

Seven persons, who were previously positive for SARS CoV-2 from June to September 2020 where no N501Y mutations were present in the region, were tested for reinfections by the same molecular methods as previously mentioned. Results of the rapid methods showed that all the 7 persons were re-infected with a variant having both D614G and N501Y mutations. The patients information and results of reinfections are shown in Table 1. The results revealed that one out of 7 reinfections carried the wildtype N501 variant (Case No.2); these were confirmed by DNA sequencings. While other 6 persons were re-infected with N501Y mutated variants which are present in the UK, South Africa and Brazil variants.

Table 1: SARS CoV-2 reinfection cases confirmed by the rapid methods
| Cases | Initial infections | Reinfections mutations | Patients clinical information |
|-------|-------------------|------------------------|-----------------------------|
| 1     | PCR positive on 10/09/2020 | D614G and N501Y mutations On 22/04/2021 | Age= 43, male, no comorbidities. First infection: asymptomatic with normal CBC and acute phase parameters. Second infection: severe dry cough, malaise for three weeks. Loss of taste and smell. |
| 2     | D614G and wildtype N501 on 10/07/2020. Sequencings: MW897353.1 | D614G and wildtype N501 on 10/02/2021. Sequencings: MW897354 | Age=35, female, obese. First infection: was feeling tired, fever, headache for 5 days and loss of taste and smell. Second infection: feeling tired, fever, and headache for 4 days. |
| 3     | PCR positive on 10/07/2020 | D614G and N501Y mutations On 24/04/2021 | Age=55, male, hypertension, diabetes and obesity. First infection: malaise, fever. Second infection, malaise, hypoglycaemia and diarrhoea. |
| 4     | PCR positive on 10/07/2020 | D614G and N501Y mutations On 24/04/2021 | Age=26, female, no comorbidities. First and Second infection: fever, tiredness, sore throat, headache, loss of taste and smell. |
| 5     | PCR positive on 25/06/2020 | D614G and N501Y mutations On 26/04/2021 | Age=41, male, obese. First infection: Myalgia, fever, headache, loss of appetite. High platelet and WBC count. Second infection: Tiredness and headache. |
| 6     | PCR positive on 10/07/2020 | D614G and N501Y mutations On 26/04/2021 | Age= 54, female, hypertension, diabetes and obesity. First infection: Dry cough, myalgia, fever, head ache, loss of appetite. Second infection: Dry cough, Tiredness and fever. |
| 7     | PCR positive on 10/07/2020 | D614G and N501Y mutations On 26/04/2021 | Age= 34, female, Asthma, First and Second infection: malaise, sore throat, headache. |

**Discussion**

This study has reported a SARS CoV-2 reinfection using rapid inexpensive techniques that can used to make differences between reinfections and persistent infections. The first case reported in this study was confirmed by the rapid tests and DNA sequencings in Kurdistan Region of Iraq. In addition, theses rapid methods, which can detect both D614G and N501Y mutations, were applied to 7 clinical samples. Out of 7 persons, one was suspected to be either re-infected or suffered from persistent infections because she carried only D614G mutations in both initial and second infections as confirmed by DNA sequencings too. However, she seemed to be re-infected rather than persistent infection as the time between both
infections were more than 6 months. The other 6 individuals carried both D614G and N501Y mutations; the latter was not present in the first infection that suggested reinfections. The re-infected patients includes different ages with both comorbidities and no comorbidities suggested that reinfections with the UK variant may occur in various ages without comorbidities.

SARS CoV-2 reinfections were previously rare in the world as in a study conducted in approximately 9 thousands of positive samples in USA from December 2019-November 2020, only 63 samples (0.7%) were reported as reinfections, which were linked with low antibody responses in the initial infection (17). However, recent research has reported 58 out of 1300 suspected reinfections among about 700,000 positive individuals in India (18). Likewise, 138 out of 28,875 positive cases were reported as reinfections in Denmark (19). An ecological research conducted in the UK concluded no evidence of increasing rate of reinfections with the UK variant (b.1.1.17) which were confirmed by whole genome sequencings (20). During writing of this manuscript, several cases of SARS CoV-2 reinfections were reported in USA, Italy, Columbia, Brazil and Luxemburg, in which their second infections were due to B.1.1.7, B.1.1.7, B.1.1.269, P1 and B.1.351 variants (21–24).

For how long antibodies persisted in previously infected persons is uncertain. However, studies suggested that antibodies may remain for approximately 6 months (3). The persistence of antibodies varies from an individual to another or it depends on the severity of the disease or the type of the variants. The antibody status of the first case was performed a day of the reinfection indicated that his adaptive immunity had no response yet and results showed that both IgG and IgM were negative. This suggested that the re-infected person has had no protective antibodies to prevent the reinfection. Limitations of the current study were lack of checking antibodies in all cases. Despite the small number of samples, this study reported reinfections with SARS CoV-2 N501Y mutant.

Conclusions

This research explores SARS CoV-2 reinfections using rapid low cost methods and reported first reinfections with a SARS CoV-2 N501Y mutant variant in Kurdistan region of Iraq. Further study is required to apply these methods in large number of samples. This will open our understandings towards the epidemiology and reinfections of the virus.

Declarations

Acknowledgements

We are grateful for the individuals who gave clinical samples to this study.

Contributors Both authors contributed equally to the entire work.

Funding No funds are available
Competing interests: None declared.

Conflicting interest: None declared

References

1. Brehm TT, Pfefferle S, von Possel R, Kobbe R, Nörz D, Schmiedel S, Grundhoff A, Olearo F, Emmerich P, Robitaille A, Günther T, Braun P, Andersen G, Knobloch JK, Addo MM, Lohse AW, Aepfelbacher M, Fischer N, Schulze zur Wiesch J, Lütgehetmann M. 2021. SARS-CoV-2 Reinfection in a Healthcare Worker Despite the Presence of Detectable Neutralizing Antibodies. Viruses 13:661.

2. Choudhary MC, Crain CR, Qiu X, Hanage W, Li JZ. 2021. SARS-CoV-2 Sequence Characteristics of COVID-19 Persistence and Reinfection. medRxiv 2021.03.02.21252750.

3. Stokel-Walker C. 2021. What we know about covid-19 reinfection so far. BMJ 372:1–2.

4. CDC. 2020. Common Investigation Protocol for Investigating Suspected SARS-CoV-2 Reinfection.

5. Al-Jaf SMA, Niranji SS, Mahmood ZH. 2021. Rapid, inexpensive methods for exploring SARS CoV-2 D614G mutation. medRxiv https://doi.org/10.1101/2021.04.12.21255337.

6. Al-Jaf SMA, Niranji SS. 2021. Rapid detection of SARS CoV-2 N501Y mutation in clinical samples. medRxiv https://doi.org/10.1101/2021.04.17.21255656.

7. Banada P, Green R, Banik S, Chopoorian A, Streck D, Jones R, Chakravorty S, Alland D. 2021. A Simple RT-PCR Melting temperature Assay to Rapidly Screen for Widely Circulating SARS-CoV-2 Variants. medRxiv Prepr Serv Heal Sci 2021.03.05.21252709.

8. Durner J, Burggraf S, Czibere L, Tehrani A, Watts DC, Becker M. 2021. Fast and cost-effective screening for SARS-CoV-2 variants in a routine diagnostic setting. Dent Mater 37:e95–e97.

9. Sandoval Torrientes M, Castelló Abietar C, Boga Riveiro J, Álvarez-Argüelles ME, Rojo-Alba S, Abreu Salinas F, Costales González I, Pérez Martínez Z, Martín Rodríguez G, Gómez de Oña J, Coto García E, Melón García S. 2021. A novel single nucleotide polymorphism assay for the detection of N501Y SARS-CoV-2 variants. J Virol Methods 114143.

10. Parry J. 2020. Covid-19: Hong Kong scientists report first confirmed case of reinfection. BMJ 370:m3340.

11. Costa AOC, Neto H de CA, Nunes APL, de Castro RD, de Almeida RN. 2021. Covid-19: Is reinfection possible? EXCLI J 20:522–536.

12. Simmonds P, Williams S, Harvala H. 2021. Understanding the outcomes of COVID-19 - does the current model of an acute respiratory infection really fit? J Gen Virol 102.

13. Davies NG, Jarvis CI, van Zandvoort K, Clifford S, Sun FY, Funk S, Medley G, Jafari Y, Meakin SR, Lowe R, Quaife M, Waterlow NR, Eggo RM, Lei J, Kolttai M, Krauer F, Tully DC, Munday JD, Showering A, Foss AM, Prem K, Flasche S, Kucharski AJ, Abbott S, Quilty BJ, Jombart T, Rosello A, Knight GM, Jit M, Liu Y, Williams J, Hellewell J, O’Reilly K, Chan Y-WD, Russell TW, Procter SR, Endo A, Nightingale ES, Bosse NI, Villabona-Arenas CJ, Sandmann FG, Gimma A, Abbas K, Waites W, Atkins
KE, Barnard RC, Klepac P, Gibbs HP, Pearson CAB, Brady O, Edmunds WJ, Jewell NP, Diaz-Ordaz K, Keogh RH, Group CC-19 W. 2021. Increased mortality in community-tested cases of SARS-CoV-2 lineage B.1.1.7. Nature https://doi.org/10.1038/s41586-021-03426-1.

14. Challen R, Brooks-Pollock E, Read JM, Dyson L, Tsaneva-Atanasova K, Danon L. 2021. Risk of mortality in patients infected with SARS-CoV-2 variant of concern 202012/1: matched cohort study. BMJ 372.

15. Hussein NR, Musa DH, Ibrahim N, Sidiq Z, Saleem M, Naqid IA. 2021. COVID-19 Reinfection in a Nurse Working in Emergency Hospital in Duhok City, Kurdistan Region of Iraq. Asian Journal of Case Reports in Medicine and Health.

16. Hussein NR, Musa DH, Naqid IA, Sidiq M, Saleem Z, Ibrahim N. 2020. The First Case of COVID-19 Reinfection in Duhok City, Kurdistan Region of Iraq: A Case Report. J Kermanshah Univ Med Sci 24.

17. Qureshi AI, Baskett WJ, Huang W, Lobanova I, Naqvi SH, Shyu C-R. 2021. Re-infection with SARS-CoV-2 in Patients Undergoing Serial Laboratory Testing. Clin Infect Dis https://doi.org/10.1093/cid/ciab345.

18. Mukherjee A, Anand T, Agarwal A, Singh H, Chatterjee P, Narayan J, Rana S, Gupta N, Bhargava B, Panda S. 2021. SARS-CoV-2 re-infection: Development of an epidemiological definition from India. Epidemiol Infect https://doi.org/10.1017/S0950268821000662.

19. Hansen CH, Michlmayr D, Gubbels SM, Mølbak K, Ethelberg S. 2021. Assessment of protection against reinfection with SARS-CoV-2 among 4 million PCR-tested individuals in Denmark in 2020: a population-level observational study. Lancet 397:1204–1212.

20. Graham MS, Sudre CH, May A, Antonelli M, Murray B, Varsavsky T, Kläser K, Canas LS, Molteni E, Modat M, Drew DA, Nguyen LH, Polidori L, Selvachandran S, Hu C, Capdevila J, Hammers A, Chan AT, Wolf J, Spector TD, Steves CJ, Ourselin S, Koshy C, Ash A, Wise E, Moore N, Mori M, Cortes N, Lynch J, Kidd S, Fairley DJ, Curran T, McKenna JP, Adams H, Fraser C, Golubchik T, Bonsall D, Hassan-Ibrahim MO, Malone CS, Cogger BJ, Wantoch M, Reynolds N, Warne B, Maksimovic J, Spellman K, McCluggage K, John M, Beer R, Afifi S, Morgan S, Marchbank A, Price A, Kitchen C, Gulliver H, Merrick I, Southgate J, Guest M, Munn R, Workman T, Connor TR, Fuller W, Bresner C, Snell LB, Patel A, Charalampous T, Nebbia G, Batra R, Edgeworth J, Robson SC, Beckett AH, Aanensen DM, Underwood AP, Yeats CA, Abudahab K, Taylor BE, Menegazzo M, Clark G, Smith W, Khakh M, Fleming VM, Lister MM, Howson-Wells HC, Berry L, Boswell T, Joseph A, Willingham I, Jones C, Holmes C, Bird P, Helmer T, Fallon K, Tang J, Raviprakash V, Campbell S, Sheriff N, Blakey V, Williams L-A, Loose MW, Holmes N, Moore C, Carlile M, Wright V, Sang F, Debebe J, Coll F, Signell AW, Betancor G, Wilson HD, Eldirdiri S, Kenyon A, Davis T, Pybus OG, du Plessis L, Zarebski AE, Raghwani J, Kraemer MU, Francois S, Attwood SW, Vaslyeva TI, Escalera Zamudio M, Gutierrez B, Torok ME, Hamilton WL, Goodfellow IG, Hall G, Jahun AS, Chaudhry H, Hosmillo P, Pinckert ML, Georgiana I, Moses S, Lowe H, Bedford L, Moore J, Stonehouse S, Fisher CL, Awan AR, BoYes J, Breuer J, Harris KA, Brown JR, Shah D, Atkinson L, Lee JC, Storey N, Flaviani F, Alcolea-Medina A, Williams R, Vernet G, Chapman MR, Levett LJ, Heaney J, Chatterton W, Pusok M, Xu-McCrae L, Smith DL, Bashton M, Young GR, Holmes A, Randell PA, Cox A, Madona P, Bolt F, Price J, Mookerjee S, Ragonnet-Cronin M, Nascimento FF,
RM, Brown R, Wyles M, Whiteley M, Zhang P, Gallis M, Louka SF, Constantinidou C, Unnikrishnan M, Ott S, Cheng JKJ, Bridgewater HE, Frost LR, Taylor-Joyce G, Stark R, Baxter L, Alam MT, Brown PE, Aggarwal D, Cerda AC, Merrill T V, Wilson RE, McClure PC, Chappell JG, Tsoleridis T, Ball J, Buck D, Todd JA, Green A, Trebes A, MacIntyre-Cockett G, de Cesare M, Alderton A, Amato R, Ariani C V, Beale MA, Beaver C, Bellis KL, Betteridge E, Bonfield J, Danesh J, Dorman MJ, Drury E, Farr BW, Foulser L, Goncalves S, Goodwin S, Gourtovaia M, Harrison EM, Jackson DK, Jamrozy D, Johnston I, Kane L, Kay S, Keatley J-P, Kwiatkowski D, Langford CF, Lawniczak M, Letchford L, Livett R, Lo S, Martincorena I, McGuigan S, Nelson R, Palmer S, Park NR, Patel M, Prestwood L, Puethe C, Quail MA, Rajatileka S, Scott C, Shirley L, Sillitoe J, Spencer Chapman MH, Thurston SA, Tonkin-Hill G, Weldon D, Rajan D, Bronner IF, Aigrain L, Redshaw NM, Lensing S V, Davies R, Whitwham A, Liddle J, Lewis K, Tovar-Corona JM, Leonard S, Durham J, Bassett AR, McCarthy S, Moll RJ, James K, Oliver K, Makunin A, Barrett J, Gunson RN. 2021. Changes in symptomatology, reinfection, and transmissibility associated with the SARS-CoV-2 variant B.1.1.7: an ecological study. Lancet Public Heal https://doi.org/10.1016/s2468-2667(21)00055-4.

21. Novazzi F, Baj A, Genoni A, Spezia PG, Colombo A, Cassani G, Zago C, Pasciuta R, Gasperina DD, Ageno W, Severgnini P, Dentali F, Focosi D, Maggi F. 2021. SARS-CoV-2 B.1.1.7 REINFECTION AFTER PREVIOUS COVID-19 IN TWO IMMUNOCOMPETENT ITALIAN PATIENTS. J Med Virol jmv.27066.

22. Staub T, Arendt V, Lasso de la Vega EC, Braquet P, Michaux C, Kohnen M, Tsobo C, Abdelrahman T, Wienecke-Baldacchino A, Francois J-H. 2021. Case series of four re-infections with a SARS-CoV-2 B.1.351 variant, Luxembourg, February 2021. Euro Surveill 26:2100423.

23. Marquez L, Koy T, Spinler JK, Luna RA, Tocco L, Fasciano L, Dunn J, Campbell JR. 2021. Reinfection with Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) B.1.1.7 variant in an immunocompromised adolescent. Infect Control Hosp Epidemiol 1–6.

24. Ramírez JD, Muñoz M, Ballesteros N, Patiño LH, Castañeda S, Rincón CA, Mendez C, Oliveros C, Perez J, Márquez EK, Ortiz F de los S, Correa-Cárdenas CA, Duque MC, Paniz-Mondolfi A. 2021. Phylogenomic evidence of reinfection and persistence of sars-cov-2: First report from Colombia. Vaccines 9.