



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.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



Active viral shedding in a vaccinated hospitalized patient infected with the delta variant (B.1.617.2) of SARS-CoV-2 and challenges of de-isolation



Abeer N. Alshukairi^{a,b,*}, Awad Al-Omari^{b,c}, Jaffar A. Al-Tawfiq^{d,e,f}, Sherif A. El-Kafrawy^{g,h}, Mai M. El-Daly^{g,h}, Ahmed M. Hassan^g, Arwa A. Faizo^{g,h}, Thamir A. Alandijany^{g,h}, Ashraf Dada^{b,i}, Mohammed F. Saedi^a, Fatma S. Alhamlan^j, Mohammad K. Al Hroub^k, Ismael Qushmaq^a, Esam I. Azhar^{g,h}

^a Department of Medicine, King Faisal Specialist Hospital and Research Center, Jeddah, Saudi Arabia

^b College of Medicine, Alfaisal University, Riyadh, Saudi Arabia

^c Department of Critical Care, Dr Sulaiman Al Habib Medical Group, Riyadh, Saudi Arabia

^d Infectious Disease Unit, Specialty Internal Medicine, Johns Hopkins Aramco Healthcare, Dhahran, Saudi Arabia

^e Infectious Disease Division, Department of Medicine, Indiana University School of Medicine, Indianapolis, IN, USA

^f Infectious Disease Division, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, USA

^g Special Infectious Agents Unit-BSL3, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia

^h Department of Medical Laboratory Sciences, Faculty of Applied Medical Sciences, King Abdulaziz University, Saudi Arabia

ⁱ Department of Pathology and Laboratory Medicine, King Faisal Specialist Hospital and Research Center, Jeddah, Saudi Arabia

^j Department of Infection and Immunity, King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia

^k Department of Infection Control and Hospital Epidemiology, King Faisal Specialist Hospital and Research Center, Jeddah, Saudi Arabia

ARTICLE INFO

Article history:

Received 26 February 2022

Received in revised form 16 April 2022

Accepted 21 April 2022

Keywords:

Vaccine

Delta variant

Viral shedding

COVID-19

ABSTRACT

In the era of SARS-CoV-2 variants and COVID-19 vaccination, the duration of infectious viral shedding and isolation in post vaccine breakthrough infections is challenging and depends on disease severity. The current study described a case of SARS-CoV-2 Delta variant pneumonia requiring hospitalization. The patient received two doses of BNT162b2 COVID-19 vaccines, and he had positive SARS-CoV-2 viral cultures 12 days post symptom onset. The time between the second dose of vaccine and the breakthrough infection was 6 months. While immunosuppression is a known risk factor for prolonged infectious viral shedding, age and time between vaccination and breakthrough infection are important risk factors that warrant further studies.

© 2022 The Author(s). Published by Elsevier Ltd on behalf of King Saud Bin Abdulaziz University for Health Sciences.

CC_BY_NC_ND_4.0

Background

In the pre-era of SARS-CoV-2 variants and COVID-19 vaccination, the duration of infectious viral shedding and isolation was ten days in patients with mild to moderate COVID-19 disease, twenty days in severe disease and several months in immunocompromised patients [1]. Recently, in the era of SARS-CoV-2 variants and COVID-19 vaccination, the guidelines of isolation of COVID-19 were revised by Centers for disease control and prevention and the duration of isolation of COVID-19 patients who received two doses of vaccines was

limited to five days in mild disease and extended to 10 in moderate disease and 20 days in severe disease [2]. A recent study described the duration of infectious viral shedding in breakthrough SARS-CoV-2 Delta variants infections in ambulatory patients with mild disease and showed that 9 out of 10 patients (90%) had negative viral cultures within 10 days of symptoms onset [3]. There are limited studies describing the duration of infectious viral shedding of post vaccine Delta variants breakthrough infections in hospitalized patients. The current study described the viral culture results of a patient who received two doses of BNT162b2 COVID-19 vaccine and was hospitalized with Delta variant COVID-19 pneumonia, six months post second dose of vaccine, in a tertiary care center in Jeddah, Saudi Arabia. The challenges of de-isolation for hospitalized and vaccinated patients infected with Delta variants were discussed.

* Correspondence to: Adult Infectious Diseases Section, Department of Medicine King Faisal Specialist Hospital and Research Center, Jeddah PO BOX 40047, Jeddah 21499, Saudi Arabia.

E-mail address: abeer.alshukairi@gmail.com (A.N. Alshukairi).

This research was approved by the institutional research board. The patient was consented and agreed to participate in the study.

Methods

Serial swabs were collected for SARS-CoV-2 PCR and viral cultures. Whole genome sequence was performed on one of the samples. SARS-CoV-2 PCR, whole genome sequence and viral culture were performed as previously described [4,5].

1. -SARS-CoV-2 PCR

Abbott Real-time SARS-CoV-2 EUA test was used for the diagnosis of COVID-19 (Abbott Park, Illinois, USA). The test is authorized by the U.S. FDA. The RNA extraction was performed on the Abbott m2000sp while the DNA amplification on Abbott m2000rt platform. The assay detects dual targets genes RdRp and N-genes with a detection limit of 1 copy per mL.

2. -SARS-CoV-2 viral culture

Cell line and SARS-CoV-2 culture

Vero E6 cells were maintained in Dulbecco's modified Eagle medium (DMEM) with 10% fetal bovine serum (FBS). A human SARS-CoV-2 clinical patient isolate (SARS-CoV-2/human/SAU/85791C/2020, Genbank accession number: MT630432) was used in all experiments as the positive control. Experiments were performed at the Special Infectious Agents Unit (SIAU) Biosafety level 3 facility at King Fahd Medical Research Center (KFMRCC), King Abdulaziz University (KAU), Jeddah, Saudi Arabia.

Detection of replicating SARS-CoV-2

Collected samples were diluted at 1:10 dilution in DMEM with 10% FBS, inoculated on Vero E6 cells in 6 well plates in duplicates, and incubated for 1hr at 37 °C. Inoculum was then removed and replaced with 2 mL DMEM with 2% FBS and incubated for 3 days at 37 °C or until cytopathic effect (CPE) was observed in 85–90% of cells of the positive control samples.

3. - Whole genome sequencing

The patient's nasopharyngeal sample was sent to the microbial genomics laboratory for SARS-CoV-2 whole-genome sequencing at the Department of Infection and Immunity, King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia. The samples were subjected to nucleic acid extraction using a MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit (Cat No. A42352, Thermo Fisher Scientific; MA, USA). All positive samples for SARS-CoV-2 were converted to cDNA using SuperScript™ IV VIL0™ Master Mix (ThermoFisher Scientific, USA). The cDNAs were amplified using the Ion AmpliSeq™ SARS-CoV-2 Insight Research Assay as indicated by the manufacturer's instructions. Amplified products were ligated with unique barcode adapters using the Ion Xpress Barcode Adapters 1–16 kit (ThermoFisher Scientific, USA) and purified with 1.5 × volume of Agencourt AMPure XP Reagent (Beckman Coulter, USA). Library was built and normalized to 33 pM using nuclease-free water, and up to 16 libraries were equally pooled for further processing. Pooled libraries were used as a template input for emulsion PCR and enrichment of template-positive particles using the Ion Chef automated system with the Ion 510 Kit-Chef kit (ThermoFisher Scientific, USA) according to the manufacturer's instructions. The obtained data were primarily processed (base calling, base quality recalibration, alignment, assembly, and variant calling) with Torrent Suite Server, version 5.12 (ThermoFisher Scientific, USA). De novo assembly of the contigs was performed using the assembly Trinity plugin (v1.2.1), and consensus sequences of each sample were

generated using the IRMA plugin (v1.2.1). Variant call files were analyzed on the COVID19AnnotateSnpEff plugin to identify and annotate variants with public and private databases.

Case presentation

The patient was an 81-year-old male patient with a history of chronic obstructive pulmonary diseases and obstructive sleep apnea on home oxygen therapy (1–1.5 liters/minute) and nocturnal BiPAP. He was not maintained on any immunosuppressive medication. He received two doses of BNT162b2 COVID-19 vaccine, the second dose was six months prior to his admission. He did not have previous infection with COVID-19. He presented with fever, cough and dyspnea of two days duration. His Chest X ray showed bilateral lung infiltrates. He was diagnosed as a case of COVID-19 pneumonia based on a positive SARS-CoV-2 PCR of a nasopharyngeal swab (Ct value 10). He was started on remdesivir for 5 days, dexamethasone for 10 days and ceftriaxone for 14 days. His sputum culture grew pan susceptible *Klebsiella pneumoniae*. During his hospitalization, he was maintained on low flow oxygen through a nasal cannula (3 l/minute). He did not require intensive care admission. During his admission, 12 days post symptoms onset, a follow up SARS-CoV-2 PCR was performed, as his clinical status was back to his baseline, and he was requesting to be discharged home and to be de-isolated. The SARS-CoV-2 PCR remained positive with Ct value of 9. The infectious diseases team were concerned to de-isolate him based on low Ct value SARS-CoV-2 PCR despite his clinical improvement. Multiple follow up SARS-CoV-2 PCR tests were performed to determine the time of de-isolation (Table 1). He was considered recovered on day 20 post symptoms onset based on symptoms rather than SARS-CoV-2 PCR results as he remained clinically stable and his SARS-CoV-2 PCR remained positive with low Ct values until day 19. Unfortunately, only one sample was available for viral culture, 12 days post symptom onset and it was positive. Most samples were sent for the national laboratory of ministry of health to include them in the whole genome sequence surveillance and they could not be retrieved for the current research project.

Discussion

The current study discussed the duration of post vaccine SARS-CoV-2 Delta Variant viral shedding in a patient hospitalized with COVID-19 pneumonia. He received two doses of BNT162b2 COVID-19 vaccines vaccine, he was infected six months post second vaccine dose and the viral culture was positive 12 days post symptom onset.

A recent review article evaluated the impact of age, vaccination status and time between the second dose of vaccine and breakthrough infection on the duration of SARS-CoV-2 viral shedding [6].

In Singapore, using SARS-CoV-2 PCR Ct values as a surrogate marker of viral shedding, vaccinated hospitalized patients infected with Delta variants had a rapid decline in viral load compared to hospitalized unvaccinated patients infected with Delta variants, 6.6 days versus 9.3 days [7]. In United Kingdom, although vaccination status and Delta variants did not affect SARS-CoV-2 peak viral load, old age (more than 50 years) was associated with an increased in peak viral load. In addition, individuals with high peak viral load had

Table 1

Serial PCR and Ct values in relation to days post symptoms onset. Viral culture was available on day 12 post symptoms onset only.

Days post-symptoms	SARS-CoV-2 PCR result	Viral culture
2 days	Ct 10.46	Not available
8 days	Ct 7.53	Not available
12 days	Ct 9.33	positive
19 days	Ct 12.86	Not available

a slow viral decline and clearance [8]. Sidner et al. described the differences in viral clearance based on SARS-CoV-2 PCR testing and viral culture among vaccinated patients infected with Delta and non-Delta variants in the ambulatory setting [3]. Compared to individuals infected with non-Delta variants, those infected with Delta variants had longer duration of viral shedding by SARS-CoV-2 PCR (13.5 versus 4.5 days) and viral culture (7 versus 4 days). In addition, compared to individuals vaccinated less than 3 months, those vaccinated more than 3 months had a longer duration of positive SARS-CoV-2 PCR (13.5 versus 3 days) and viral culture (7 versus 3 days). Among a cohort of patients infected with Delta variants, there was no difference in the duration of viral shedding between vaccinated and partially vaccinated individuals using SARS-CoV-2 PCR testing (13 days) and viral culture (5 days) [9]. The findings from the current study of persistent positive viral cultures for more than 10 days in hospitalized patients infected with Delta variants and defining the duration of isolation of these patients is challenging and requires further evaluation. While immunosuppression is a known risk factor for prolonged infectious viral shedding, determining duration between the last dose of vaccine and the breakthrough infection is important to predict the duration of infectious viral shedding and time of de-isolation. In view of waning post-vaccine antibody responses within six months in patients 65 years of age or older [10], patient may have more prolonged infectious viral shedding. In the current study, whereby a patient had a breakthrough infection six months post second vaccine dose and his viral culture was still positive 12 days post symptom onset. De-isolation may need to be extended more than 10 days for patients with moderate disease when the last dose of vaccine is more than six months even in the absence of immunosuppression.

Serial SARS-CoV-2 PCR and viral cultures were not performed in the current study, in addition to lack of SARS-CoV-2 serological testing post vaccine. A large sample size study of hospitalized and vaccinated patients infected with SARS-CoV-2 variants is needed to confirm our findings. In the era of emergence of new variants and the different vaccination doses of individuals including boosters, the duration of isolation needs future studies in both ambulatory and hospitalized patients. In a recent study evaluating the efficacy of two versus three doses of COVID-19 mRNA vaccines during the Delta and Omicron surge, the Ct values of Delta and Omicron COVID-19 samples were higher among patients who received three mRNA vaccine compared to those who received two doses indicating a lower SARS-CoV-2 viral load among patients who had boosters compared to those who had only two vaccine doses [11]. Determining a reliable and a rapid surrogate marker for infectious viral shedding such as rapid antigen tests requires future studies as PCR testing is associated with prolonged viral shedding and may not reflect infectious viral shedding while viral cultures are time consuming and needs special research laboratories [12].

Conclusions

The current case study showed prolonged SARS-CoV-2 infectious viral shedding for 12 days post symptoms onset in a hospitalized patient with COVID-19 variant (Delta) pneumonia, who was more than 65 years old and was vaccinated 6 months prior to infection. A test-based approach may be required to end isolation. The ideal de-isolation test needs to be studied.

Ethics approval and consent to participate

This research was approved by the institutional research board, King Faisal Specialist Hospital and Research Center, Jeddah, Saudi Arabia.

Funding

The whole genome sequence work is funded by King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia: COVID19 grant 2200009 This research was also funded by Jameel Fund for Infectious Disease Research and Innovation in Saudi Arabia grant (8-3) 2120191.

Acknowledgment

We would like to thank our patient who contributed to this research. The authors acknowledge the generous charitable donation from the Late Sheikh Ibraheem Ahmed Azhar in the form of reagents and supplies as a contribution to the scientific research community. The authors would like to thank the funding support of King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia (grant 2200009) and Jameel Fund for Infectious Disease Research and Innovation in Saudi Arabia (grant (8-3) 2120191). We appreciate the technical support of King Abdulaziz University, Jeddah, Saudi Arabia. Special thanks to Dr Ashraf Dada for his tremendous efforts to support our research projects during COVID-19 pandemic in the molecular laboratory of King Faisal Specialist Hospital and Research Centre, Jeddah, Saudi Arabia. We would like to thank Professor Stanley Perlman, University of Iowa Distinguished Chair, USA for reviewing the manuscript.

Conflict of Interest

None.

Contribution

All authors contributed equally to this research; study concept, data acquisition, data analysis, performing the experiment, writing and reviewing the manuscript.

References

- [1] Badu K, Oyebola K, Zahouli JZB, Fagbamigbe AF, et al. SARS-CoV-2 viral shedding and transmission dynamics: implications of WHO COVID-19 discharge guidelines. *Front Med* 2021;8:1–11.
- [2] CDC. Ending isolation and precautions for people with COVID-19: interim guidance. (<https://www.cdc.gov/coronavirus/2019-ncov/hcp/duration-isolation.html>).
- [3] Sidner MJ, Boucau J, Gilbert RF, et al. Duration of viral shedding and culture positivity with postvaccination SARS-CoV-2 delta variant infections. *JCI Insight* 2022;7(2):e155483.
- [4] Alshukairi AN, El-Kafrawy SA, Dada A, et al. Re-infection with a different SARS-CoV-2 clade and prolonged viral shedding in a hematopoietic stem cell transplantation patient. *Int J Infect Dis* 2021;110:267–71.
- [5] Almaghrabi RS, Alhamlan FS, Dada A, et al. Outcome of SARS-CoV-2 variant breakthrough infection in fully immunized solid organ transplant recipients. *J Infect Public Health* 2022;15:51–5. <https://doi.org/10.1016/j.jiph.2021.11.021>
- [6] Amanatidou E, Gkiouliava A, Pella E, et al. Breakthrough infections after COVID-19 vaccination: insights, perspectives and challenges. *Metab Open* 2022;14:100180<https://doi.org/10.1016/j.metop.2022.100180>
- [7] Chia PY, Ong SWX, Chiew CJ, et al. Virological and serological kinetics of SARS-CoV-2 Delta variant vaccine breakthrough infections: a multicenter cohort study. *Clin Microbiol Infect* 2022;28(4):612.e1–7.
- [8] Singanayagam A, Hakki S, Dunning J, et al. Community transmission and viral load kinetics of the SARS-CoV-2 delta (B.1.617.2) variant in vaccinated and unvaccinated individuals in the UK: a prospective, longitudinal, cohort study. *Lancet Infect Dis* 2022;22:183–95.
- [9] Salvatore, P.P., Lee, C.C., Sleweon, S., et al., 2021. Transmission potential of vaccinated and unvaccinated persons infected with SARS-CoV-2 Delta variant in a federal prison, July-August 2021. *medRxiv* doi.org/10.1101/2021.11.12.21265796.
- [10] Levin EG, Lustig Y, Cohen C, et al. Waning immune humoral response to BNT162b2 COVID-19 vaccine over 6 months. *N Eng J Med* 2021;385(24):e84<https://doi.org/10.1056/NEJMoa2114583>. Epub 2021 Oct 6.
- [11] Accorsi EK, Britton A, Fleming-Dutra KE, et al. Association between 3 doses of mRNA COVID-19 vaccine and symptomatic infection caused by the SARS-CoV-2 omicron and delta variants. *JAMA* 2022;327:639–51. <https://doi.org/10.1001/jama.2022.0470>
- [12] Binnicker MJ. Can testing predict SARS-CoV-2 infectivity? The potential for certain methods to be surrogates for replication-competent virus. *J Clin Microbiol* 2021;59(11):1–8.